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STUDIES ON THE FORMATION OF CELLULAR SUBSTANCES DURING BLOOD CELL PRODUCTION

By

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INTRODUCTION

The blood corpuscles circulating in the blood stream have a limited span of life, and new ones are continuously formed in the bone marrow. To maintain a constant number of blood cells in the blood stream a considerable production is necessary; in the adult man c. 100 mill. erythrocytes are formed per minute. In principle the formation of new blood cellular substance takes place from a relatively small and constant number of stem cells which produce a large number of mature blood cells by means of division and maturation through morphologically characteristic stages. The formation of mature blood cells from the stem cells in the bone marrow involves the two fundamental processes of cell renewal, partly *growth* by increase in size and division and partly *differentiation* into a functionally specialized tissue cell. The purpose of the present investigation is to analyse quantitatively the processes involved in growth and differentiation during blood cell renewal.

As the cells at different stages of development are intermixed in the bone marrow, the individual phases in the processes of growth and differentiation are not open to ordinary quantitative methods of analysis. The data which exist are chiefly of a morphological nature, which do not permit any quantitative or qualitative interpretations of the processes during the formation of new cellular substance.

One possibility of attacking these questions is to investigate by quantitative microchemical methods the processes within the individual cell. *I. e.* to analyse the cytochemical processes which are linked up with the new-formation and differentiation of the cellular substances and are responsible for cellular growth and maturation.

Growth, whether it takes place by division or by enlargement of the cell, can be defined as the new-formation of the fundamental

substances of the cell. The latter usually consist mainly of proteins. The formation of proteins can thus be considered the quantitatively dominant process during cell growth.

A starting-point for the investigation of the cytochemical processes during blood cell renewal can be obtained from the works in the last decade which have been carried out on the endocellular mechanism for the protein new-formation. A survey of these results is given in Chap. 2, Part I.

In Chap. 3 an account is given of the theoretical basis and the method of cytochemical analysis of living bone marrow cell material. Chap. 4 gives an account of the analysis data of cellular material from normal hematopoiesis in mammals and man.

With these analyses as the basis the last two chapters in Part I deal with the endocellular growth processes during the new-formation of blood cells and with their cytogenetic regulation mechanism.

In Part II is examined the relationship of the cellular growth processes to the specific cell differentiation processes, *i. e.* from a cytochemical point of view the relationship between the new-formation of cellular proteins during growth to the changes of these proteins into their specific functional structures. Particularly suitable for studying this is the differentiation process during erythropoiesis, the synthesis of hemoglobin. This can be relatively easily followed by means of quantitative cytochemical methods of analysis.

Finally, with the results from Part I and II as a background, the disturbances in the cell-physiological processes during hematopoiesis in the case of certain pathological conditions are dealt with in Part III.

PART I.

CHAPTER 1.

The Development of the Cells of the Blood in the Adult.

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The development of blood cells in the adult individual has been the subject of a vast number of investigations and theories so that an account of all of them does not seem possible within reasonable limits. Only some general principles will be referred to below. For the rest reference should be made to the comprehensive surveys published by Bunting (1922), Sabin (1922), Doan, Cunningham and Sabin (1925), Downey (1927), Naegeli (1931), Doan (1932), Maximow (1932), Whitby and Britton (1935), Isaacs (1937), Osgood and Seaman (1944), and others.

A. The Morphology of Hematopoiesis.

In the normal adult mammals the blood cell formation is localized solely in the bone marrow, where the original tissue is to be regarded as an undifferentiated mesenchyme. The reticular cells in it are relatively small and have a slightly basophilic cytoplasm without any other specific substances observable in stained preparations. The nucleus is poor in chromatin. By division and maturation these cells give rise to the first stages of free blood cells, the blood stem cells. This transformation can be observed as an aggregation in the cytoplasm of mitochondria and basophilic substances. The essential process in the maturation of blood

cells is the formation of hemoglobin and granula. Simultaneously with the formation of hemoglobin or granula the cytoplasmic-basophilia of the blood stem cell decreases. These processes are continuous, but depending on the varying proportions of these substances together with changes in the nuclear picture and the size of the cell, certain maturation stages in the development of the red and white blood cells have been defined and named.

The cytology of the development into mature blood cells from the stem cells is well known. Nevertheless the hematological literature is difficult to interprete mainly owing to the fact that the nomenclature is not uniform. Many different names have been used for the same type of cell, and many different kinds of cells have been given the same name. Therefore it appears necessary to indicate here the terminology with synonyms and references employed by the present author.

With regard to the main group of the development series of the white blood cells (granulocytopoiesis) the nomenclature of FERRATA and PAPPENHEIM has been employed.

1. The myeloblast, the immature, primitive stem cell. [Hemocytoblast (MAXIMOW 1927), Lymphoidocyte (PAPPENHEIM 1905), Granuloblast (OSGOOD and ASHWORTH 1937)].

2. The promyelocyte, in which the first signs of the formation of granula appear [Progranulocyte (OSGOOD 1937), Myelocyte A (SABIN 1928)].

3. The myelocyte with granula and dense chromatin structure in oval nucleus. (Granulocyte, Myelocyte B.) This cell by way of a poorly defined intermediate stage, the metamyelocyte (the metagranulocyte, Myelocyte C), where the nuclear shape has passed over to the rod shape, finally matures into

4. The definitive granulocyte (leukocyte, lobocyte).

The classification of the maturation phases during the development of the red blood cells (erythropoiesis) presents greater difficulties than the classification of the white, owing to the absence of clear morphological changes such as the appearance of granula, lobing of the nuclei, etc. The most generally employed nomenclature is based on the stainability of the cytoplasm with acid and basic stains (HELLY 1910, MAXIMOW 1927).

1. The stem cell is called pro-erythroblast [Erythrogonie (HELLY 1910, ELLERMAN 1920)].

2. Basophilic erythroblast [early erythroblast (TURNBULL 1936)].

3. Polychromatic erythroblast, in which the appearance of hemoglobin can be observed (late erythroblast).

4. Orthochromatic erythroblast with entirely acidophilic cytoplasm [normoblast (TURNBULL)], which loses the nucleus and matures into the erythrocyte circulating in the blood.

B. The Cell Physiology of Hematopoiesis.

1. The Formation of Granula.

According to HOLMGREN and WILANDER 1937, certain basophilic granula are akin to mucoitin sulphuric acid and can be specifically demonstrated in the cell as metachromatically stainable. Other granula can be demonstrated in the cell with peroxidase reagents, and will probably consist at least in part of substances resembling peroxidase (AGNER, 1941). A substance isolated by AGNER, verdoperoxidase, has an isoelectric point at pH 10 and probably corresponds to certain of the eosinophilic granula of the granulocytes. The data concerning the formation of these substances during granulocytopoiesis are of a purely morphological character, however: they probably appear intracellularly from certain precursors (progranulation) and, judging by the cytological picture, not until the cell has attained a certain degree of maturity. (WINKLER, 1907, GRÄFF, 1922, etc.)

2. The Formation of Hemoglobin.

It has been shown in works by SHIPLEY, SABIN, DOAN, CUNNINGHAM, SLONIMSKI, MURRAY, etc. that the formation of hemoglobin takes place in cells which have originated from undifferentiated mesenchymal tissue. In chicken embryo, for instance, hemoglobin formation takes place in the primitive red cells at such an early embryonal stage of development that a direct co-operation of other tissue-cells in the synthesis of hemoglobin is out of the question.

Investigations into the synthesis of hemoglobin have been mainly of a nutrition-physiological character: the works of WHIPPLE, HOOPER and ROBSCHEIT-ROBBINS, which show that the formation of Hb is greatly influenced by foodstuff factors: iron, certain amino acids, etc., the investigations of MINOT and MURPHY into the liver principle, CASTLE's observations that essential factors for a normal synthesis of hemoglobin are intimately associated with the ventricular digestion, etc., the im-

portance of the vitamins, especially ascorbic acid (FOKINA, LEVERTON). All these observations show that the synthesis of hemoglobin is a sensitive mechanism intimately influenced by a multitude of external factors.

There has been much speculation by morphologists as to the endocellular course of the formation of hemoglobin. Naturally it has been linked to the general morphological cell changes during erythropoiesis. Thus, *e. g.* LOELE assumed that hemoglobin was formed in the erythroblast by diffusion to the spongioplasm of nuclear substance, the so-called parachromatin, where it was combined with amino bases existing in the cytoplasm. Later, cytochemists such as BRACHET, DUSTIN, etc. could prove, by the ribonuclease technique, nucleic acids in the cytoplasm of the erythroblast, and assumed that ribose nucleic acids are in some way linked up with the synthesis of hemoglobin.

An interesting, purely chemical investigation of the formation of hemoglobin of the immature red blood corpuscles was carried out by BURMESTER in 1937. On the basis of the well defined chemical and physico-chemical properties of hemoglobin and its derivatives and employing chemical and spectro-photometric methods, he investigated in chickens the composition of red blood corpuscles in different stages of maturity in respect to hemoglobin derivatives (hemochromogen, hematoporphyrin, oxyhemoglobin free and bound iron, etc.). Sufficient quantities of different maturation-stages were secured for analysis by means of differential centrifugation of chicken blood, in which the blood cells had been made immature by repeated blood tappings. The chief results appear to show that the formation of hemoglobin begins in an early stage of development — from a morphological point of view — during erythropoiesis. He was able to distinguish a series of porphyrin derivatives as probable precursors of hemoglobin, the earliest being akin to but not identical with hematoporphyrin. Except in the most immature stages he found that relatively early the iron was bound to some ill defined precursors of hemoglobin. Although BURMESTER's results do not indicate anything as to the quantitative courses during erythropoiesis, they suggest that the synthesis of hemoglobin is connected with metabolic processes which take place mainly endocellularly parallel to the maturation of the red blood corpuscles.[1]

[1] Recently (1946) SHEMIN and RITTENBERG have demonstrated by the use of N^{15}, that glycine is the nitrogenous precursor of the protoporphyrin of hemoglobin.

3. Growth.

In the hematological literature the interest in the other principal process during the formation of new blood cells, growth, has been chiefly centred on the problem of which cell stage in the development series is responsible for the main part of the new formation in the normal adult individual. Histologists and tissue cultivators especially (Maximow, Doljanski, etc.) consider that the development of each newly formed blood cell originates from the immature blood stem cell, which by means of division produces maturing cells. Clinicians, on the other hand, hold the view that under normal conditions the new formation takes place exclusively by divisions of more mature cells such as the myelocyte (Rohr). They find their chief support for this in the frequency of mitosis in a fixed and stained preparation of bone marrow; for example c. 50 % of all mitoses within the granulocytopoiesis belong to the myelocyte stage.

The chief cause of these conflicting views has apparently been *the difficulty of evaluating the growth state of an individual cell.* The frequency of mitoses, which has often been employed, does not afford an adequate measure of this.

A number of general cytological characteristics such as cytoplasm-basophilia, large nucleolar mass, fine-meshed chromatin network, etc. have certainly been set in connection with the immaturity of the cell, but there have been no possibilities of correlating these phenomena with the cell-physiological growth processes.

CHAPTER 2.

The Endocellular Organization for the Formation of Protein.

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As mentioned above in the introduction, growth can be defined, whether it takes place by division or by enlargement of the cell, as the new-formation of the fundamental substances of the cell. As the latter consist mainly of protein substances the formation of proteins can be regarded, in general, during cell growth as the quantitatively dominating process. For the first part of this investigation, dealing with the growth processes of hematopoiesis the experiences of the last ten years in respect to the endocellular organisation for protein-formation can thus provide a starting-point. It has been shown, chiefly by CASPERSSON and co-workers that an intensive cellular protein formation takes place in the presence of nucleic acid metabolic processes.[1] A survey of these results will be given below.

[1] The nucleic acids are divided into two groups according to the sugar they contain, ribose and ribodesose nucleic acids. Apart from the sugar component, the basic unit in nucleic acid (nucleotide) consists of purine base or pyrimidine base and phosphoric acid. The two groups of nucleic acids differ also in a pyrimidine base component; ribose nucleic acid contains uracil instead of its methylated derivative thymine in ribodesose nucleic acid.

Chemical data collected from recent years show the universal distribution of ribose and ribedesose nucleic acid both in plant and animal cells. For references see LEVENE and BASS, 1931, MIRSKY, 1943, DAVIDSSON and WAYMOUTH, 1944, and GREENSTEIN, 1944.

A. Methods.

To attack the cell-physiological problems dealing with the relationship between structure, chemical composition and function during the growth processes, microchemical methods are necessary which permit a *quantitative* determination of the groups of substances which constitute the chief cellular constituents within parts of a cell in the living or inappreciably altered condition. In other words methods are necessary which can determine quantities of 10^{-8}—10^{-12} g without greatly affecting the state of the material.

In 1936—1940 T. Caspersson developed a microspectrographic method which was based on the high, selective light absorption of the nucleic acids in the central ultraviolet region. This absorption maximum at 2600 Å is due to the purine and pyrimidine bases in the nucleotides, is practically identical for both ribose and ribodesose nucleic acids, and is hardly affected by external factors such as pH. The absorption coefficient at 2600 Å is of such an order of magnitude that, with layer thicknesses corresponding to the diameter of the cells, and with the concentration of these substances in the cell, the light absorption lies within measurable range. The technique developed has rendered it possible to determine 10^{-12} g of nucleic acid within an area of 0.2—0.5 μ in diameter.

The aromatic amino-acids in the cell protein, tyrosine, tryptophane and phenylalanine, also exhibit selective light absorption in the same wave length range. The absorption band at 2800 Å which is shown by the majority of protein substances, is caused by these amino acids, and therefore in certain cases the amount of protein can be calculated from the light absorption data (see below p. 48).

When the accuracy in the microspectrographic measurement was brought down to 1 % (Caspersson, 1940) in many places in different cells a small but distinct displacement of the protein band in relation to the nucleic acid was found. The same displacement was also found in 6 different histone preparations (Caspersson, 1940). Comparisons with Schenck's chemical data (Schenck, 1932) on amino acid distribution in tissues from embryos of different ages showed a correlation between the content of diamino acids and the appearance of the displacement (Caspersson and Thorell, 1941). The mechanism for the spectral shift is not clear. Histone preparations entirely free of nucleic acid did not show the spectral shift (Mirsky, Mirsky and Pollister 1942).

Another procedure used for diamino acid localization is founded on

the observations of Chapman, Greenberg, Schmidt 1927, who found that at very acid reactions certain dyes reacted with the free amino groups of proteins in stoichiometric relations and could be used for determination of the free basic groups (Hydén 1943). In each case where the above-mentioned shift in protein absorption band was found, the staining reaction showed an especially high content of diamino acids (see Caspersson 1947). The observation of the spectral shift can thus be used as an indication of the presence of relatively large amounts of diamino acids.

For the localization of nucleic acid it is also desirable to have a suitable phosphate method, and such a procedure has been worked out by Norberg (1942). With this procedure amounts as small as 10^{-7} mg can be determined, that is, about 10^{4-5} times less than the usual micromethods. However, during the recent years a new analytical method has been developed, based on absorption- microspectrography in the X-ray spectral range, which allows the determination of a large number of elements without combustion of the tissues (Engström 1946). For phosphorus the new procedure is considerable more sensitive than the colorimetric procedure.

Finally, there should also be mentioned the Feulgen reaction which has been used to qualitatively discriminate between ribodesose nucleic acids and ribose nucleic acids (Feulgen and Rossenbeck 1924).

Thus, mainly by means of microspectrographic determinations of the light absorption spectrum in the central ultraviolet (3200—2400 Å), it is possible to determine within individual cells the amount of nucleic acids and aromatic amino acids with relatively great accuracy, the sum of other amino acids less accurately, and also of determining roughly the distribution of diamino acids.

B. Principal Results.

These methods have rendered possible the study of the metabolism of nucleic acids and proteins within individual cells, especially during the growth processes. The results have shown on the whole, that the self reproduction system which functions for the new-formation of cellular proteins consists essentially of *protein combined with nucleic acid.*

During cell division (Caspersson 1936, Caspersson and Schultz 1938) considerable changes were found in the nuclear ribodesose nucleic acid concentration and distribution. During

the prophase, ribodesose nucleic acids were accumulated on the chromosome elements. Analysis of living cell nuclei (CASPERSSON 1939) during the meiosis from early leptotene to diplotene showed a total increase in ribodesose nucleic acids immediately before the division of the chromosomes. These observations led to the conclusion that *the reproduction of the gene elements during cell division is conditioned by the presence of ribodesose nucleic acid.*

In 1938—1939, in the root-tips of Allium, CASPERSSON and SCHULTZ found high concentrations of ribose nucleic acid in the cytoplasms of the growth zone. On the other hand, the cells towards the base of the root had a purely tyrosine-tryptophan absorption spectrum. The circumstance that the new-formation of proteins is one of the main processes in rapidly growing cells, and that the cytoplasms of these cells are characterized by high concentrations of ribose nucleic acid made it probable that in these growing cells ribose nucleic acid was in some way linked up with the protein generation of the cytoplasm. (CASPERSSON and SCHULTZ 1939.) Later investigations, 1939—1945, on a large material: embryonal tissue, protein secreting glands, cancer tissue, growing egg cells, yeast cells, blood cells etc. showed the general applicability of this principle: *an intense protein generation in the cytoplasm is always correlated with an increase in the cytoplasmic concentration of ribose nucleic acid.* (CASPERSSON 1941, CASPERSSON and BRANDT 1941, CASPERSSON and THORELL 1941, CASPERSSON, LANDSTRÖM-HYDÉN and AQUILONIUS 1941, CASPERSSON and SANTESSON 1942, HYDÉN 1943, HYDÉN and CASPERSSON 1945, LANDSTRÖM et al. 1941, MALMGREN, CASPERSSON and THORELL 1945, NORBERG 1942, THORELL 1944, 1945, THORELL et al. 1945, THORELL and WILTON 1945, THORELL and WISING 1944.)

C. The Connection Between Nucleus and Cytoplasm During the Formation of Cellular Proteins.

1. The Nucleolus.

Analysis of nucleolus and cytoplasm in rapidly growing sea urchin eggs showed that both these cell-organellas contain large amounts of ribose nucleic acids (SCHULTZ, CASPERSSON and AQUILONIUS 1940). Similarly in embryonal cells, blood cells, nerve cells, etc. a clear parallelism was found between nucleolar mass containing ribose nucleic acid and cytoplasmic nucleic acid (CASPERSSON

and THORELL 1941, HYDÉN 1943, THORELL 1944). Studies on this material showed that, apart from ribose nucleic acid, the nucleolus also contained considerable amounts of protein substances abundant in diamino acids. From these observations emerged a nucleolus definition based on the cytochemical properties of the nucleolar mass: *A nuclear organella in protein-generating metazoan-cells which consists mainly of ribose nucleic acid and also protein substances abundant in diamino acids.*

The relation between the nucleolar apparatus of the nucleus and the nucleotide-protein formation system of the cytoplasm was studied in detail on ganglion cells from Lophius piscatorius (HYDÉN 1943). A concentration gradient of protein substances rich in diamino acids from the nucleolus towards the part of the nuclear membrane where the cytoplasm's formation of ribose nucleic acid took place, indicated a migration of basic proteins from the nucleolus to the nuclear membrane, where the formation of the cytoplasmic ribose nucleic acid was induced.

2. The Chromatin.

Studies by CASPERSSON and SCHULTZ (1938—1940) on Diptera material (Drosophila) demonstrated a connection between the ribodesose nucleic acid metabolism of the chromosomes on the one hand and the ribose nucleic acid metabolism linked up with the new-formation of cellular protein on the other. In the case of a translocation between X and the 4th chromosome, where the rupture in the latter lay in a heterochromatic part of the chromosome, an increase in the concentration of nucleic acid was found in those of the X-chromosome bands which through the rearrangement came to be nearest to the heterochromatic parts (CASPERSSON and SCHULTZ, 1938, SCHULTZ, 1941). Further, it was observed that the amount of the cytoplasmic nucleic acid in the egg was related to the amount of heterochromatin. If an extra Y-chromosome was introduced into an XX-hen, the amount of ribose nucleic acid in the ovum of the XXY-individual showed an increase in comparison with that of the XX-hen.

These observations may be summarized as follows: The heterochromatic regions in the Drosophila chromosomes apparently affect the nucleic acid metabolism and therewith the protein metabolism:

a) in genes in the same chromosome, b) in other chromosomes in the same nucleus, and c) in the cytoplasm.

The connection between the heterochromatin and the nucleolus-cytoplasmic nucleic acid mechanism emerged from studies on Drosophila and Chironomus material (CASPERSSON 1940, 1941). The analysis showed that the production of nucleolar substance abundant in ribose nucleic acid and diamino acids, proceeded directly from the heterochromatic chromosome regions.

On cellular material of the higher animals also it could be shown that certain chromatin parts which diverged from the chief part of the chromatin were associated with the cytoplasmic protein forming mechanism. (CASPERSSON and THORELL 1941, CASPERSSON and SANTESSON 1942, HYDÉN 1943, THORELL 1944.) In strongly growing cells these chromatin parts were characterized chemically during the interphase by their production of ribose polynucleotides and protein substances, containing considerable amounts of diamino acids, which were aggregated into a nucleolus. This chromatin was called the nucleolus-associated chromatin (CASPERSSON and THORELL 1941, THORELL 1944) and in a limited number of cases where it was possible to compare this chromatin with the genetically defined heterochromatin the conceptions seem to coincide. The name "nucleolus-associated chromatin" was chosen to avoid the name heterochromatin, as the cytological and genetical definition of the latter in ordinary nuclei still seems to be very difficult and to some extent a matter of choice.

D. Survey of the Endocellular Mechanism for the Formation of Cellular Proteins.

The above-mentioned observations on very different material indicated, that a quite identical endocellular organization functioned in all types of cytoplasmic protein formation. This can be visualized in the following manner (fig. 1).

A certain part of the chromatin, the nucleolus-associated chromatin (A), produces substances composed of ribose nucleic acids and proteins rich in diamino acids during the telophase-interphase. These substances accumulate and form the main bulk of the nucleolus (B). From the nucleolus they diffuse towards the nuclear membrane (D), on the outside of which an intensive production of ribose nucleic acids takes place. At the same time the amount of cytoplasmic proteins increases. This protein generation is apparently in some way linked up with the nucleic acid changes.

In the study of the growth processes in different metazoan tissue cells it is of course very rarely possible to measure *e. g.* the gradient from nucleolus to nuclear membrane. This is due to the small size of the cell. In the picture of a cell in intensive protein production, however, two phenomena are so evident that they cannot be missed, no matter how small the cell is, and these are the changes in the nucleolus and the appearence of the large amounts of ribose nucleic acid in the cytoplasm. An example will be given below.

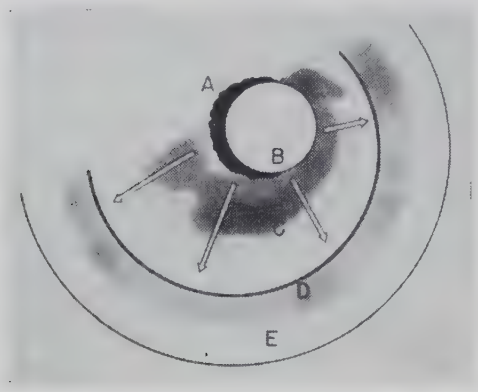
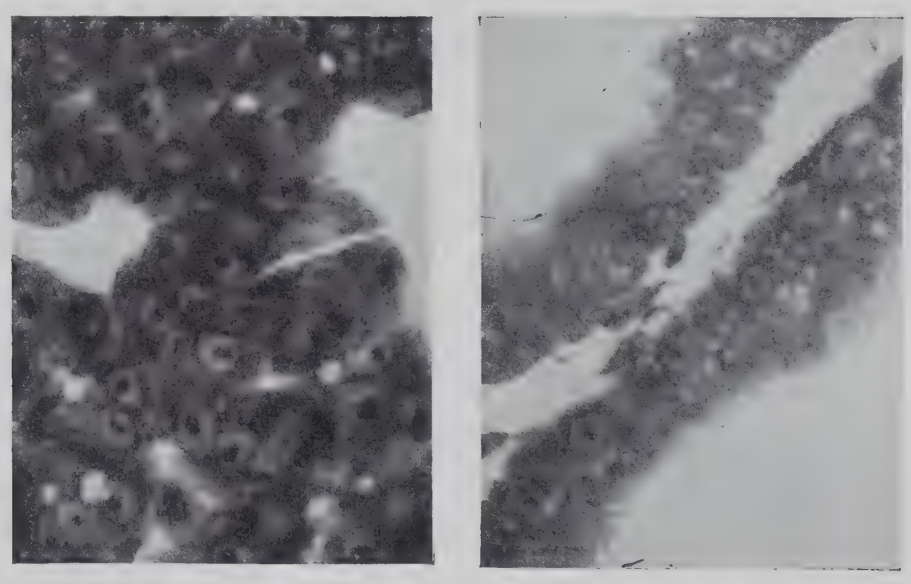


Fig. 1. Diagrammatic representation of the cytoplasmic protein-forming system, according to Caspersson et al. A, nucleolus-associated chromatin; B, nucleolus. The arrows indicate the migration of basic proteins (C) from the nucleolus-associated chromatin, direct or via the nucleolus, to the nuclear membrane, D. In the vicinity of the latter the formation of cytoplasmic ribose nucleic acid, E. and cytoplasmic protein proceeds.

The most distinct protein generation occurs during the embryonal growth. Fig. 2 a shows embryonal liver cells from a chicken embryo of the 6th day of incubation. The large nucleolar mass is evident and the absorption spectrum shows very high concentrations of ribose nucleic acids in the cytoplasm, *i. e.* the cells have a cytoplasmic protein formation system in intense function according to the diagram in fig. 1.



a b

Fig. 2. Microphotographs at wavelength 2570 Å of: a) rapidly growing liver cells from a 6-day chicken embryo, b) adult kidney cells. 550 X. (From Caspersson and Thorell 1941.)

Fig. 2 b shows adult kidney cells. In the adult tissues the generation of cytoplasmic proteins is of quite another order of magnitude and the organellas of the cellular protein-forming mechanism are correspondingly little developed.

E. The Primary Problem of the Investigation.

Starting from these experiences of the endocellular mechanism for the formation of cellular proteins the primary question will be in investigating the growth processes of hematopoiesis: does a similar ribose nucleic acid metabolism and endocellular organization linked up with the generation of cytoplasmic proteins exist during the *growth* of the blood cells, and further, are the cytochemical effects during the continous normal new formation of blood cells in the adult organism of such an order of magnitude that they can be subjected to quantitative ultramicrochemical methods of analysis?

In this connection it should be pointed out that from the point of view of method, owing to their small dimensions, the blood cells and their precursors are not very suitable for detailed cytochemical analyses. However certain principal structures such as the nucleolus and the cytoplasm will probably be available for analysis.

CHAPTER 3.

The Procedure for Microspectrographic Analysis of Living Bone Marrow Cells.

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The claims which may be laid on a method of analysis suitable for this investigation may be summarized as follows: The method shall permit a quantitative analysis of identifiable substances within the structures of an intact and preferably living cell. Of the procedures, differing in principle, which have been worked out for cytochemical analyses, only the purely optical ones seem to satisfy these needs. With them one can most simply determine substances which have a light absorption, preferably a selective one.

The groups of substances in this investigation are firstly, the nucleic acids (see Chap. 2) connected with the processes of cell growth, and secondly the specific protein-substances connected with the differentiation-processes such as hemoglobin (see Part II). Of these the nucleic acids have selective light absorption in the ultraviolet (Dhéré, 1906, Heyroth and Loofbourow, 1931, 1934, Caspersson 1936) and the colouring matter of the blood mainly in the visible spectral range (Vierordt 1875, Cherbuliez,

1890, Vlès 1921, etc.). It is of decisive importance for the estimation of these substances in the cell by means of the light absorption analysis that the magnitude of the absorption constant and the concentration of the substance give a sufficiently accurate measurable weakening in intensity of light (in general 5—90 %), in the layer corresponding to the thickness of the cell structure. The import of these preconditions appears from the following: In a 10 μ thick layer a 2.5 % ribose nucleic acid solution at 2600 Å gives a light weakening of 60 %. 3 % hemoglobin in the same layer gives a 20 % absorption at 4047 Å. Thus in suitably chosen wave lengths the respective absorption constants permit a quantitative determination within the layer thicknesses and concentration ranges in which they occur in the cell.

Perhaps more than in the case of any other quantitative analytical procedures, correct performance of the cytochemical light absorption analysis requires strictly defined conditions both as regards the material under investigation and the technical equipment. An account of these conditions will be given below with the quantitative analysis of the cellular elements of hematopoiesis as the background.

A. Conditions for the Feasibility of the Measurements.

1. *General conditions.*

It is obviously necessary in determining the optical constants of a microscopic object with instruments which include dioptric systems, that the distribution of the intensity in the image given by the microscopic lenses corresponds to the true distribution of the intensity in the object. For a system of lenses with certain minimum apertures (> 1.0) and satisfying Abbe's sine law, Caspersson (1936) showed that true absorption measurements can be made on microscopic objects the size of which is not less than 3—4 times the wavelength used.

These results are only true if certain conditions are controlled. The aperture is of particular importance, *i. e.* all light leaving the object must participate in forming the microscopic image.

The errors in absorption measurements which are due to *the properties of the object* are mainly unspecific losses of light owing to reflection and refraction boundary surfaces with discontinuities in the refractive index. Calculations of these errors for certain forms of objects and index values were made by Caspersson in 1936.

Owing to their distribution of light absorbing substances and small size (see below) bone marrow cells present, however, such complicated optical conditions that these calculations are not directly applicable. The optical properties of these cells will therefore be dealt with below.

Measurements of the diameters of living bone marrow cells in different directions in space show that they are spherical in form (see Table 1 with text). Thus, in the calculations of unspecific loss of light from reflexion and refraction an *absorbing, light refracting sphere* can be a cell model.

Table 1.

The diameters of living bone marrow cells in different directions of space[1].

Myeloblasts		Promyelocytes		Myelocytes		Erythroblasts	
breadth μ	depth μ	breadth μ	depth μ	breadth μ	depth μ	breadth μ	depth μ
12	10	18.5	18	10	9.5	7.5	7
13	12.5	15	15	10	9	7	7
12	11	15	15	11	10	7	6.5
14.5	13	13	10	8	8	8	8
12	12.5	12	12	12	12	7	7
11	11	12	11	11	11	9	8
13.5	13	20	18	12	11	6.5	6.5
12	11.5	12	13	11	9	7	6.5
		15	14.5	10	10	9	8
		16	13	9.5	10	5	5
		13	13	11	11	5	5.5
		12	13.5	9	8	8.5	8
				10	9	8	7
				10	10	7	6.5
				12	10	6	6

The calculations aim at leading up to: 1) the intensity distribution in the image of an absorbing, *light refracting* cell sphere illuminated by a *convergent* bundle of rays. Further, 2) the intensity distribution must be calculated in the image of an absorbing *non-light refracting* sphere of the same size illuminated by a *parallel* bundle of rays. In case 2 the absorption constant can simply be deduced for all points, as the weakening in the intensity of rays with the angle of incidence φ can be calculated according to Lambert-Beer's law:

$$E = \log \frac{I_0}{I_1} = k \cdot d \cdot \cos \varphi \text{ (see fig. 3)}$$

---

[1] The cell diameter in the vertical plane ("depth") was measured by use of an achromatic lens system, inserted between the objective and the ocular lenses in a phase contrast microscope. By varying the distance between the positive and the negative lenses of the achromatic system (max. distance 10 cm), the focal distance of the objective lens and thereby the focal plane is changed ($\pm$ 10 μ).

The cell diameter in the horizontal plane ("breadth") was measured by an ocular screw-micrometer corrected for the changes of magnification caused by the above mentioned objective lens accessory.

The difference between the two diameters is on an average less than 5 ± 2 %.

where E = the extinction, I_0 = the incident light intensity, I_1 = the transmitted light intensity, k = the absorption constant, d = the diameter, and φ = the angle of incidence. From a comparison between the intensities of the images in the two cases point by point, the magnitude of the divergencies conditioned by light refraction and convergent bundle of rays will emerge.

Constants:

The absorption coefficient = 500, which at 2600 Å corresponds to a 2.5 % ribose nucleic acid solution.

Cell diameter = 10 μ.

Cell refraction index. The value was obtained in the following manner: Bone marrow abundant in cells from young animals was frozen and ground. The refraction index and the dispersion were determined in Abbe's refractometer. The total reflexion limit which corresponded to the maximum average refraction index (most unfavourable case) was employed for determining the value. $n_D = 1.374$. Dispersion $(n_F - n_C) = 63.0 \times 10^{-4}$. (Table 2.)

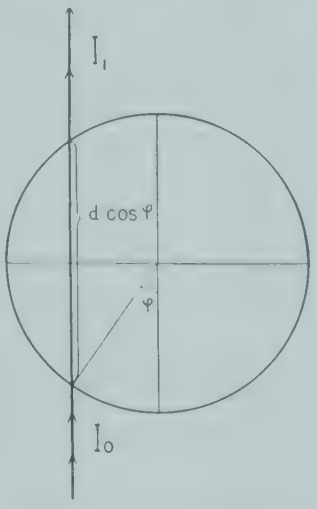


Fig. 3. Light-absorption in a non-refracting sphere, illuminated with a parallel bundle of light.

The refraction index of the surrounding medium, $n_D = 1.335$, $n_F - n_C = 62.0 \times 10^{-4}$. (Table 2.)

The aperture of the illuminating bundle of rays: $2 \times 30°$.

Table 2.

Index of refraction (n_D), estimated by the use of the Abbes refractometer at temp. 19° C.

	Tyrode saline solution	Blood serum	Bone marrow cells
n_D	$1.3350 \pm 2 \cdot 10^{-5}$ $\sigma = 4 \cdot 10^{-5}$	$1.3466 \pm 3 \cdot 10^{-5}$ $\sigma = 8 \cdot 10^{-5}$	$1.3738 \pm 20 \cdot 10^{-5}$ $\sigma = 95 \cdot 10^{-5}$

2. *Light reflection.*

In the case of sudden changes in the refractive index a light-dispersion owing to reflexion occurs. This can be calculated from Fresnel's formulae.

For these low refraction indices (see table 2) and for spheres there are calculations according to Fresnel's formulae made by Caspersson and Engström (1946),

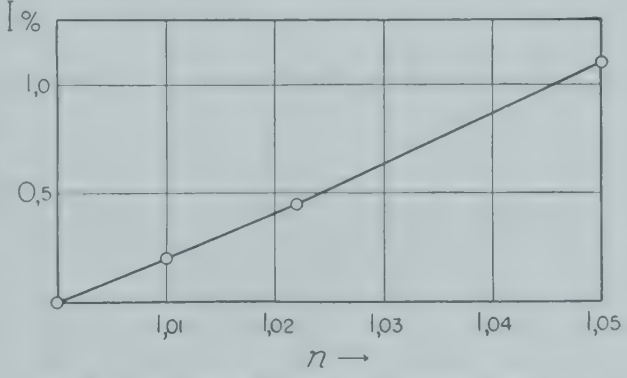


Fig. 4. The loss of light (I) due to reflection in refracting sphere, calculated according to Fresnel's formulas.

the results of which are shown in fig. 4. From this figure it emerges that the loss of light at the index quotient 1.03 will be less than 0.7 % ($E = 0.003$) and thus may be ignored. Owing to the fact that the dispersions for both media are practically the same, the loss of light by reflexion will be almost constant over the main part of the wave length range employed.

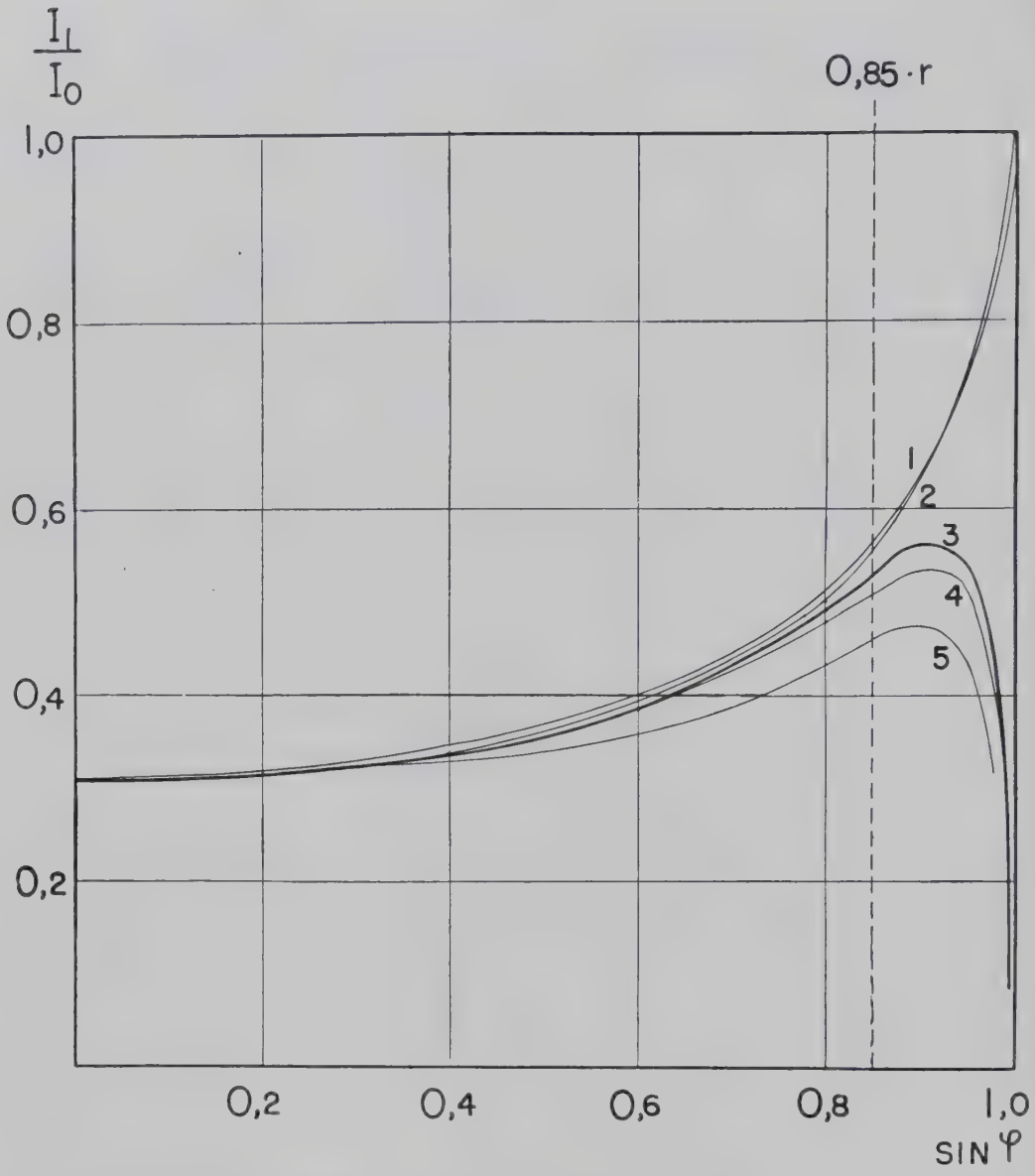


Fig. 5. The intensity distribution in the image of: 1) an absorbing, non-refracting sphere, illuminated with a parallel bundle of light, 2) an absorbing, non-refracting sphere, illuminated with a convergent bundle of light, 3) an absorbing, refracting (n = 1.03) sphere, illuminated with a convergent bundle of light, 4) an absorbing, refracting (n = 1.03) sphere, illuminated with a parallel bundle of light, and 5) an absorbing, refracting (n = 1.1) sphere, illuminated with a convergent bundle of light.

3. *Light absorption in bone marrow cells illuminated with a convergent bundle of rays.*

The factors which may condition the divergencies from Lambert-Beer's law in the intensity distribution of the image of the object are the light refraction and the convergent illumination bundle of rays (see above).

According to CASPERSSON (1936), the divergencies conditioned by the light refraction, is calculated for a refracting sphere illuminated with parallel light according to:

$$I = \frac{dx}{d\varphi} \cdot \frac{1}{r \cdot \cos \varphi} \cdot 10^{-\varepsilon \cdot 2r \sqrt{1 - \frac{\sin^2 \varphi}{n^2}}}$$

where

φ = the angle of incidence
x = the intersection of the beam with the diameter of the sphere
r = the radius of the sphere
ε = the extinction coefficient
n = the refractive index.

The calculations with $n = 1.03$, $r = 5\ \mu$ and $\varepsilon = 500$ are shown in fig. 5, curve 4. Together with curve 1 (absorbing, non-light refracting sphere) it emerges that up to $0.80 \cdot r$ the deviation caused by light refraction is in this case less than 1 %.

The deviation conditioned by the convergent bundle of rays is calculated according to CASPERSSON (1936) p. 64 from:

$$E_1 = I_0 \int_{-u}^{+u} \arccos\left(\frac{\cos u}{\cos v}\right) \cdot 10^{-\varepsilon \cdot 2r \sqrt{1 - \sin^2 \varphi \cos^2 \alpha}} \cdot d\alpha$$

where $2 \cdot u$ = the aperture angle of the illuminating light. Other symbols are given below.

The calculations for non-refracting, absorbing sphere, illuminated with an aperture of $2 \cdot 30°$, are shown in fig. 5, curve 2, which shows that the deviation over the whole image is less than 1 %.

If an absorbing, refracting sphere is illuminated with convergent light we obtain the following: The amount of energy is considered as the cone volume ABC (fig. 6). (This implies the approximation that after refraction the rays converge to one point.)

The path of light through the sphere (fig. 6):

the absorbing distance $= 2r \cos \psi$

from the law of refraction $= \sin(\varphi - \alpha) = n \sin \psi$

$$\therefore 2r \cdot \cos \psi = 2r \sqrt{1 - \frac{\sin^2(\varphi - \alpha)}{n^2}}$$

through the sinus theorem:

$$\frac{\sin(\varphi - \alpha)}{x} = \frac{\cos \alpha}{r}$$

the path of light $\therefore$

$$2r \cos \psi = 2r \sqrt{1 - \frac{\sin^2 \varphi \cdot \cos^2 \alpha}{n^2}}$$

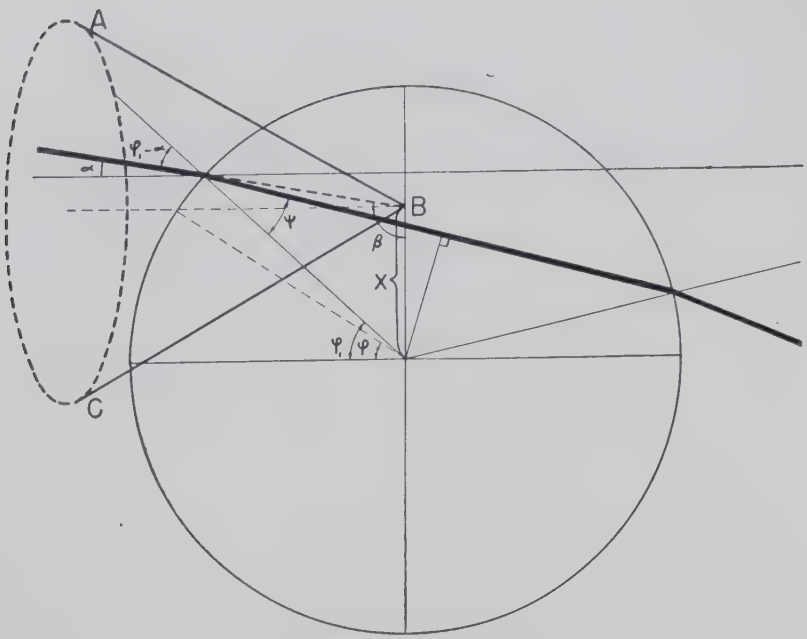


Fig. 6. Calculation of the path of light in an absorbing, refracting sphere, illuminated with a convergent bundle of light. Sphere radius = r. The side of cone = a. The rest of the symbols are seen in the figure.

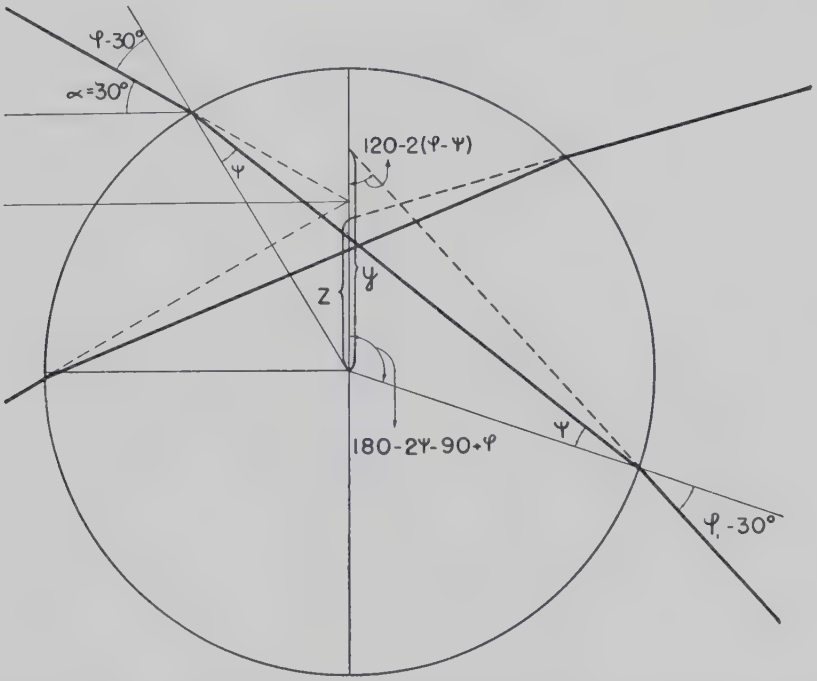


Fig. 7. Calculation of the intersections (z and y) of the illuminating light cone in the image plane.

Incident light on the objective (in curved plane):

$$E_1 = \frac{2}{3}\,a^3 \int_{-u}^{+u} \arccos\left(\frac{\cos u}{\cos \alpha}\right) \cdot 10^{-\varepsilon \cdot 2r \sqrt{1 - \frac{\sin^2 \varphi \cos^2 \alpha}{n^2}}} \cdot da$$

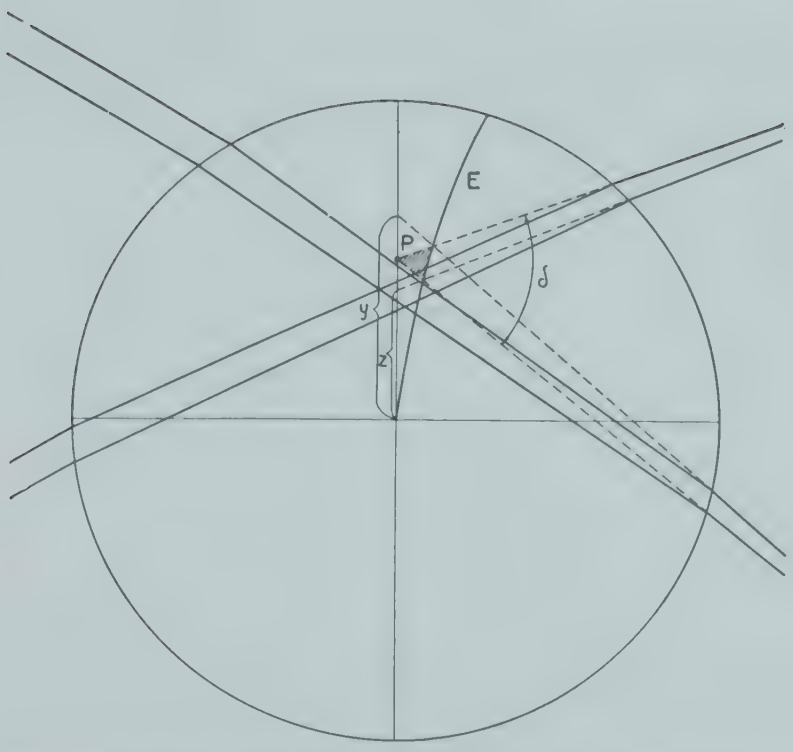


Fig. 8. Calculation of the light-intensity in the image plane (E_p) of an absorbing, refracting sphere, illuminated with a convergent bundle of light. See text.

The intersections of the cone of light with the focussing plane (fig. 7)

$$\frac{y}{r} = \frac{\sin(\varphi - 30°)}{\sin[120° - 2(\varphi - \psi)]}$$

$$\frac{x}{r} = \frac{\sin(\varphi_1 + 30°)}{\sin[60° - 2(\varphi_1 - \psi)]}$$

Light cones with apexes along surface E between y and z (fig. 8) contribute to the intensity in point P in the plane of the image. The rays from these illuminating cones form an inverted cone with the apex at P and an apex angle of δ, which amount of illumination may be expressed as a function of the mean energy of the ray components of the latter cone.

$$E_p = k \cdot \frac{\sum_{z}^{y} \cdot k \int_{-u}^{+u} \arccos\left(\frac{\cos u}{\cos \alpha}\right) \cdot 10^{-\varepsilon \cdot 2r \sqrt{1 - \frac{\sin^2 \varphi \cos^2 \alpha}{n^2}}} \cdot d\alpha}{y - z} \cdot \delta$$

$$\delta = 2(\varphi - \psi) - 60°.$$

This function calculated by means of graphic integration, gives for $n = 1.1$ an intensity distribution according to curve 5, fig. 5, and for $n = 1.03$ curve 3. It appears from the curves that in the former case the deviation from the curve 1 is considerable even at $0.7 \cdot r$, while in the latter right up to $0.85 \cdot r$ the deviation is less than 1 %.

Thus it is theoretically possible within a living bone marrow cell ($n = 1.03$) to make true absorption measurements with errors of less than 1 % within an area of $0.85 \cdot r$ (see fig. 9).

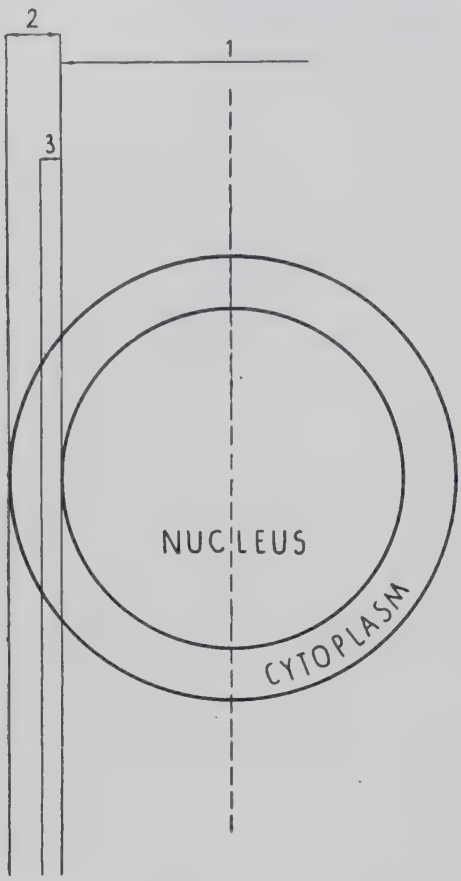


Fig. 9. Schematic representation of the optical conditions for absorption measurements in a living bone marrow cell of spherical shape. Light beam parallel to the plane of paper. Range 1 indicates the projection of the nucleus, range 2 the projection of the cytoplasm. In the latter, real absorption measurements can be made within range 3 *i. e.* from the nucleus out to 0.85 cell radius. Further out the light absorption will be disturbed by refraction due to refractive index jump from surrounding medium to the cell. For examples of measurement, see fig. 37.

B. Measurement Apparatus.

For the solution of the problems in this investigation (see above) quantitative light absorption analyses of the structures in the individual bone marrow cell are required, in ultraviolet and the visible spectral range.

The conditions are different in ultraviolet and in visible light respectively. To make the analyses of, *e. g.* the composition of the cytoplasm in the ultraviolet spectral range account must be taken into the great light absorption of the ribodesose nucleic acid-containing nucleus. In practice this brings with it that only a small area, larger than the nuclear diameter and less than $0.85 \cdot r$, is available for measurement (see above, fig. 9). Living cells are also sensitive to ultraviolet light; they are quickly cytolysed by irradiation with moderate light intensities below 3000 Å. Further

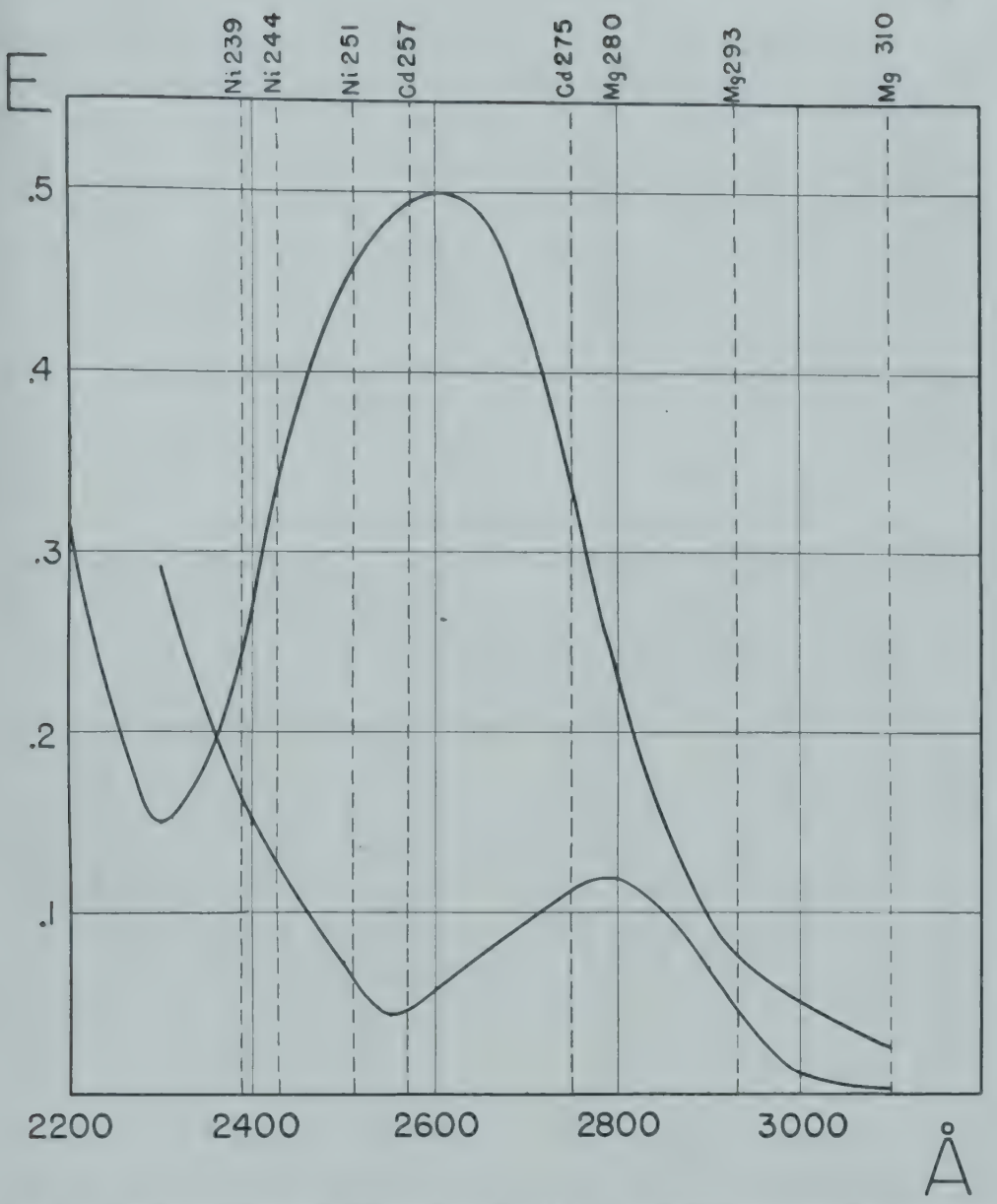


Fig. 10. The ultraviolet absorption of a 0.02 ‰ ribodesose nucleic acid solution (upper curve) compared with that of a 0.2 ‰ solution of serum albumin (lower curve). The measurements were made with a Beckmann quartz spectrophotometer. Nucleic acid preparation according to Hammarsten 1947. The serum albumin was a commercial product.

In this and the following figures the extinction is defined as $E = \log \frac{I_0}{I_1}$.

The dotted lines indicate the wavelengths used for the microspectrographical analyses (spark emission lines of Ni, Cd and Mg). See text.

it is not possible to control the adjustment manipulations, etc. directly with the eye.

On the other hand, in the visible spectral range the living cell is practically speaking optically empty, except for specific substances

e. g. hemoglobin. It is not particularly sensitive to irradiation. Adjustments, etc. can be carried out directly under the control of the eye.

These factors together condition certain differences in the technical procedure for microspectrographic analysis of living bone marrow cells in the ultraviolet and visible spectral range respectively. The description below is therefore divided up accordingly. In principle an arrangement is used in which the essential parts are a source of light, monochromator, microscope optics, and light registration device in the plane of the image.

I. Ultraviolet microspectrography.

a. *Source of light and monochromator.*

The wave length range for microspectrography in ultraviolet, where the protein substances and nucleic acids of the cell have their selective light absorption, lies between 3100 and 2400 Å. The course of the light absorption appears from fig. 10.

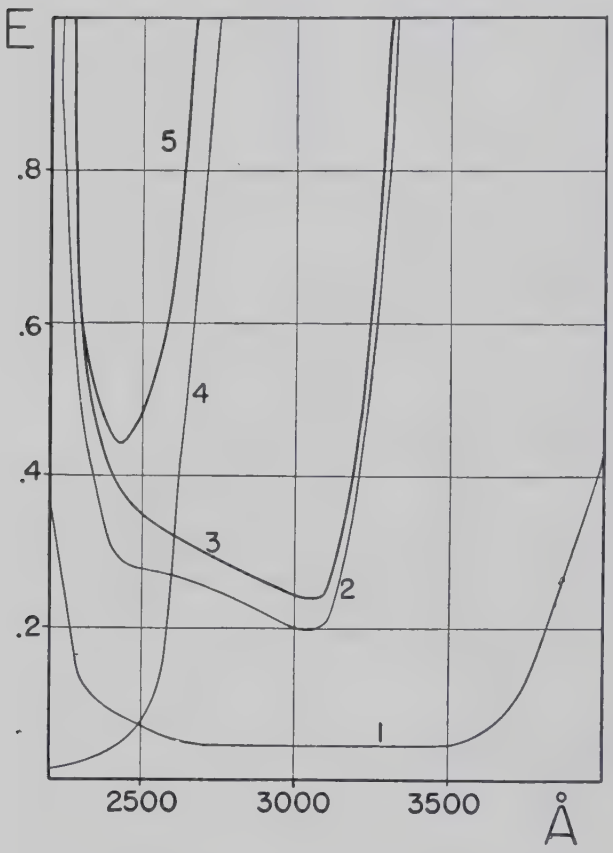


Fig. 11. Absorption of the filter substances used for further purification of the spark emission lines: 1 = 1 cm 2 M $CoSO_4$, 2 = 3 cm 2 M $NiSO_4$, 3 = curve 1 + curve 2, 4 = Cl_2 at 760 mm pressure, 10 cm, and curve 5 = curve 3 + curve 4.

By means of a rotating spark gap indicated by Köhler (1933) sufficient light intensities can be obtained in all the wave lengths indicated in fig. 10, even with the great magnifications necessary for the analysis of bone marrow cells. The effect is 3000 VA.

After the collector the light is refracted through two 60° quartz prisms filled with water, after which the spark is projected on the aperture diaphragm of the condenser (Köhler, 1893).

The steep slope of the absorption curves and the greatly reduced sensitivity of the photographic material in short wave lengths

render it necessary to use strictly monochromatic light. This is effected partly by using electrode material, the spark spectrum of which consists of well separated lines, and partly by filtering off the scattered light. This is effected with a filter according to BÄCKSTRÖM (1940): 3 cm of 2 M $NiSO_4$ and 1 cm of 2 M $CoSO_4$ (trade preparation pro analysi, purified from traces of Fe according to BÄCKSTRÖM, 1940). For the short wave nickel lines 10 cm of Cl_2 at atmospheric pressure were also introduced into the light path. The transmission data of the filters measured in spectrometer according to WARBURG-NEGELEIN are shown by fig. 11.

b. *Microscope optics.*

The condensor is a Zeiss' system in molten quartz with a maximal num. ap. of 0.8 and a focal distance of 6.8 mm, glycerine immersion. For microspectrographic purposes it is appropriate to work with a numerical aperture of 0.6. Even illumination of the object is secured if it is arranged according to the principle of KÖHLER (1893).

The objective lens is a quartz monochromat constructed by KÖHLER and v. ROHR, glycerine immersion, with a focal distance of 2.5 mm and num. ap. 0.85. With a quartz ocular 5 ×, a tube length 16 cm and 57 cm distance from the plane of the object to the intensity registration plane (plane of the image) 1000 × linear magnification is obtained.

c. *Intensity registration.*

In order to obtain correct light absorption data it is necessary to have an aperture of the measuring objective lens which is large compared with the illuminating bundle of rays. Such an optical system leads to great magnifications. However, the disadvantage of such a system is the considerable loss of light. Thus the intensity registration will work with very small amounts of light per surface unit. In this connection for objective determinations two methods are open: the photo-electric and the photographic.

In this investigation for the microspectrographic analyses in ultraviolet a method of photographic intensity determinations is employed, whereby, when using living bone marrow cells, certain technical difficulties are eliminated. In this case the advantages of the photographic method as against the direct photo-electric intensity measurements can be summarized as follows: 1) the

living bone marrow cells need only be exposed to the ultraviolet light for short periods, 2) the density of the photographic material can be determined within a very small area, whereby it is possible to measure a small definite surface in the cell object without the necessity of increasing the magnification and the incident light intensity and 3) it can easily be corrected for a slight shift in position or change in form of the living cell object, which cannot be continuously controlled directly by the eye.

As the connection between the density of the photographic material and the amount of incident light is affected by a number of factors and can be expressed in simple formulae only under very restricted conditions, it is necessary to use a special calibration system in order to obtain quantitative intensity data from the density. A number of procedures are known from the quantitative macrospectrography which — with greater or lesser modifications — can be employed within microspectrography.

Calibration with a series of different exposure times is the simplest. In this case, however, this method is none too appropriate, chiefly owing to the difficulty of securing a constant source of light, especially in ultraviolet, of accurate timing ($\pm$ 1 %), and as the photographic emulsion does not strictly follow the reciprocal law ($D = f(I \cdot t)$, where D = density, I = intensity, t = exposure time) but is differently affected by the time variation and intensity variation respectively (Schwartzschild, Kron, etc.).

The intensity variation with a constant time factor is obtained if the light is weakened by means of an absorbing wedge. In ultraviolet down to 2500 Å can be employed on quartz-glass cathode-pulverised platina or antimony (Merton, Harrison). However such a wedge is not entirely independent of wave length (Follet, 1936). For light weakening Uber 1939 used a quartz glass step-wedge of this kind with cathode pulverised platina. His data, however, indicate certain disadvantages, firstly the difficulties of calibration in the form of swinging values due to unevenness in the layer, and secondly the dependence on wave length which causes an extinction variation within 2800 — 2500 Å of 10 %.

Caspersson (1936) described a method of photographic intensity determinations for microspectrography in ultraviolet. The standard system, a neutral glass wedge, was photographed separately on each plate with a constant amount of light in the visible spectrum, since it was difficult to obtain even illumination over a considerable plate-area with the source of light employed in ultraviolet. The density curves of the plate material, *i. e.* the graphic connection between the amount of light and the density between wave lengths from Cd 2145 to Zn 3300 and in visible light was determined. Thus for a certain density value correction could be applied to the value which would be obtained if the plate was exposed and developed under exactly the same conditions as prevailed during the determination of the density curve. In

this manner intensity values are obtained with an accuracy of 10 %. The method is unsuitable for this purpose chiefly as it is unnecessarily time-consuming.

A calibration principle which has been largely employed within macrospectrography is a light-weakening rotating sector (Scheibe, 1925). It can also be employed in the case of microspectrography in ultraviolet, when the sector is rotating close to the plane of the image (Cole and Brackett, 1940). The advantages of this arrangement are: the sector can easily be made exact, and it is entirely independent of wave length, which is a great advantage especially in ultraviolet. One drawback, however, is the intermittency effect, *i. e.* intermittent exposures have not the same photochemical effect as an equivalent continuous exposure (Lumière, 1881). If the source of light is intermittent, interference may arise, which depends on the rotation speed of the sector.

O'Brien (1929) and also Webb (1933) showed that the intermittency effect is dependent on the frequency, as the effect disappears when the intermittent flashes exceed a certain critical frequency, varying with the intensity of the illumination. Webb found this critical frequency to be in the neighbourhood of 100/sec. in the case of 1 sec. exposures and only a few flashes per sec. with a 1-hour exposure. Sawyer and Vincent (1943), however, found that such a critical frequency only existed for certain photographical emulsions, especially slow ones, whereas it could not be reached in the case of other more rapid ones.

The dependence of the intermittency effect on the wave length appears to vary (Davis 1926, Weinland 1934). For instance, the intermittency effect increased in the neighbourhood of ultraviolet in the highly sensitive Eastman-Speedway, while for photomechanical plates (Eastman Process) it increased in the neighbourhood of long wave range of the spectrum. The intermittency effect also was shown to depend on which part of the density curve was utilized, *i. e.* was dependent on the total amount of incident light. The deviation, compared with an equivalent continuous exposure, increased with increased density, until the straight part of the density curve was reached ($D = 0.4$ —0.6), after which it fell (Davis) or became constant (Weinland).

With the object of investigating whether the magnitude of the intermittency error renders a rotating sector in the plane of the image less suitable as a calibration system for microspectrographic purposes, the following experiment was carried out: The intensity in the plane of the image of the above described microspectrographic equipment was changed by varying the distance from the plane of the object, and by means of a rotating sector divided into 5 steps from 10 % to 80 % absorption. The exposure time was kept constant with a precision stop watch with a circuit breaker which was connected in relay with the primary circuit of the transformer in the source of spark light. The evenness of the illumination of the field of the image was controlled on "empty" plates the density of which was determined from edge to edge in a self-registering microphotometer according to Siegbahn. Three different kinds of plates were used: Agfa's "photomechanical Platten

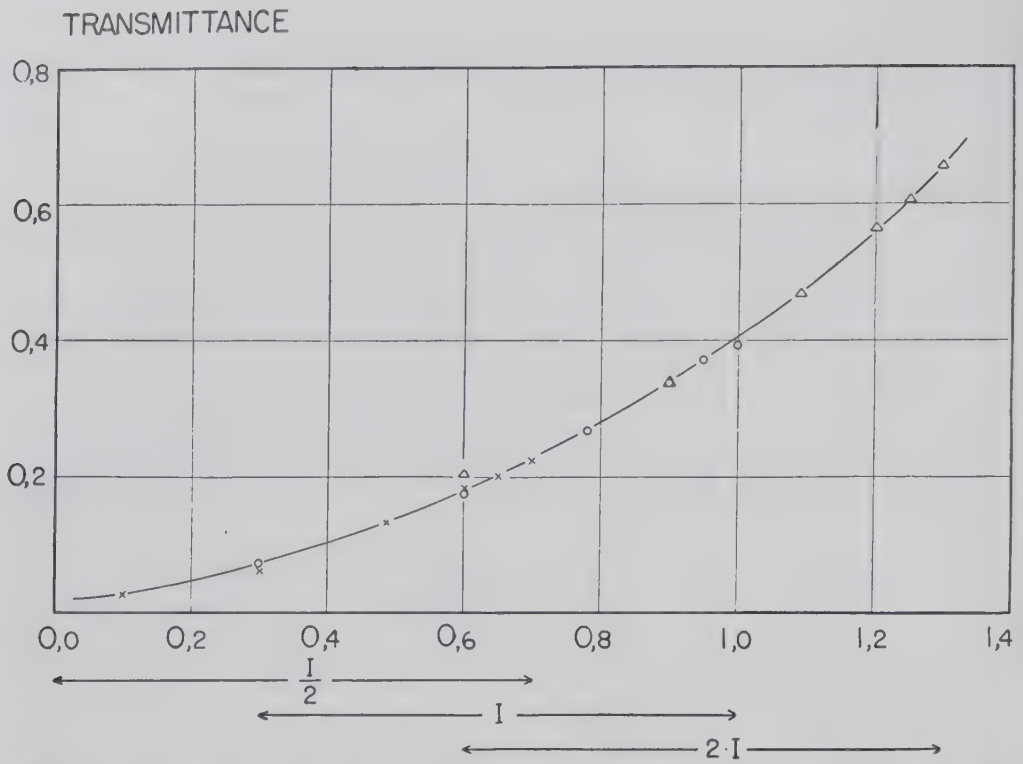


Fig. 12. The transmittance of a Process plate, the illumination of which has been changed by using a rotating sector (10, 20, 40, 60, and 80 % absorption) and by varying the distance from plate to the object plane ($\frac{1}{2}$ I, I and 2.I). It appears from the figure that the intermittency effect of the rotating sector is negligible.

B", Gevaert's Process and Imperial Process. The results shown in fig. 12 demonstrate that the deviation is negligible.

As the total exposure time varies for each of the sector steps, errors may conceivably arise owing to divergences from the reciprocal law. To investigate whether this may have any effect on the accuracy under the existing conditions, sector images were taken with constant light intensity, but with the exposure times so chosen that the maximum density varied from 0.2—0.6. The results appear in fig. 13 and show that the reproduceability of the individual determinations is $\pm$ 2 %.

With the spark device employed as the source of light (see above), fed with 500 per/sec. alternating current there are possibilities of interference. A calculation shows that in the most unfavourable cases, where the sector aperture is $2 \cdot 18°$ ($E = 1.0$) and the sparks/period only 2, there is a great risk of disturbing interference at 3000 rpm and over. At 1500 rpm the risk is less and at about 750 rpm practically nil. Thus owing to the high frequency of the spark it is possible even in the case of comparatively short exposures to adjust the revolutions of the sector in such a way that there is no disturbing interference.

Summary of c: With microspectrography of living bone marrow cells a photographic-photometric method of intensity determina-

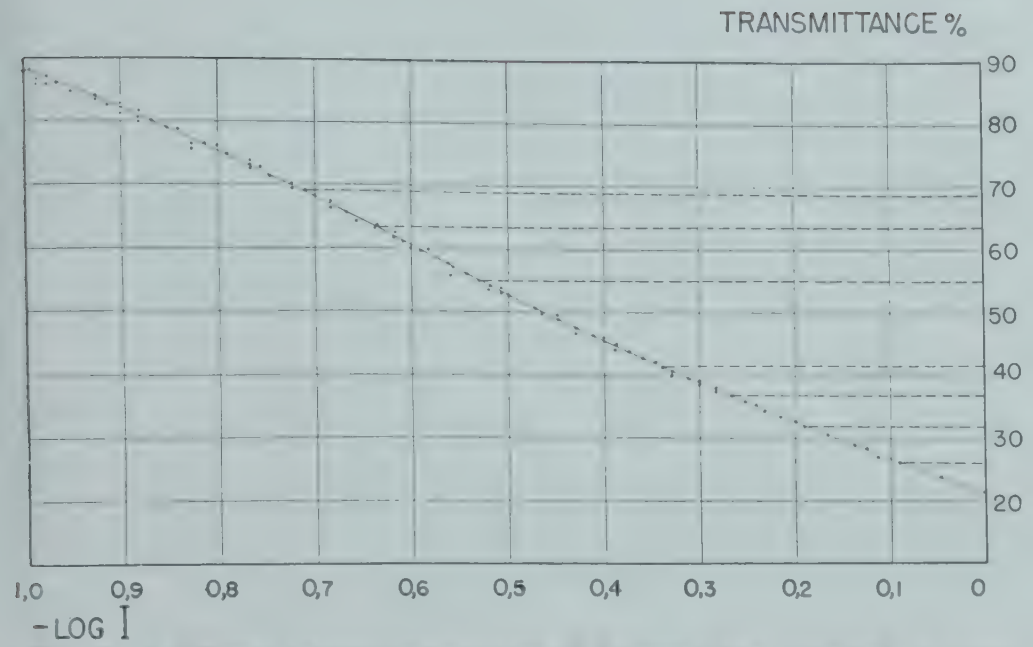


Fig. 13. 8 different transmittance curves of Process plates, obtained by photographing a rotating sector with 8 different exposure times. The curves have been made to coincide by displacements along the -log I axis. See text.

tions is to be preferred on account of the sensitivity of the cells to irradiation and the smallness of the cellular structures. An inventory of different calibration principles indicates that the most appropriate method is a sector rotating in the plane of the image. The errors which are thereby introduced do not exceed about two per cent.

d) *Procedure.*

After the bone marrow preparation has been made (see below p. 46) the cell object and the illumination is adjusted in the ultraviolet microscope with the help of KÖHLER's fluorescent ocular. The selection of cells suitable for analysis must be photographically made at wave length 2570 Å (primary current 15 A). The object should not be illuminated unnecessarily as then it is soon cytolised.

When a cell has been selected for analysis the electrode material in the spark gap is changed to Mg, the current strength reduced to 7 A and an appropriate filter combination inserted in the light path (see table 3). The exposure is made with a precision stop watch with a circuit-breaker relay-coupled to the primary circle of the spark-source of light. A homogeneous plate material should be used throughout (Process plates of Ilford, Imperial or Agfa makes).

Table 3.

Exposure scheme.

Wavelength mμ	Primary current amp.	Filter	"Net-diaphragm"[1]	Exposure time sec.
Mg 310	7	Ni — $CoSO_4$	—	2.0
Mg 293	»	»	+	2.0
Mg 280	»	»	+	1.5
Cd 275	15	»	+	3.0
Cd 257	»	»	—	1.5
Ni 251	20	Ni — $CoSO_4$ + Cl_2	—	15.0
Ni 244	»	»	—	30.0
Ni 239	»	»	—	60.0

Condenser aperture: 0.85; Objective lens: Quartz monochromat 2.5 mm; Ocular: Quartz 5X; Tube length: 160 mm; Distance from objectplane to plate: 570 mm; Photographic plates: Agfa Platten B, Imperial Process or Ilford Process. For developer and development time see text.

Metol-hydroquinone developer[2] is used at a temperature of 19° ± 0.5 (water bath). After 2 min. the developing is instantaneously interrupted with an acid fixing bath.[3]

For determinations of the exposure time under the experimental conditions it is appropriate to make a test exposure in 2930 Å, at which wave length the cell is relatively insensitive to irradiation. To reach the part of the density curve suitable for measuring, the plate should be exposed in such a way that the unweakened incident light intensity corresponds to a density of 0.5, *i. e.* the maximum density must have a transmittance of about 30 %.

If the exposure time found in 2930 Å is divided by that indicated in table 3, a factor is obtained with which exposure times for all the other wave lengths indicated in the table can be calculated.

After the exposure time has been determined, a series of microphotograms is taken in the Mg, Cd and Ni spectral lines respectively, attention being paid to the data in table 3. While this is done the rotation speed of the sector is controlled. The exchange of electrodes should be made rapidly, in order that the cell object shall be kept as intact as possible.

When the whole series from 3100 Å to 2390 Å has been taken, the plates are developed simultaneously with the developer, time

[1] Gauze-net inserted in the plane of the condenser diaphragm. This arrangement increases the exposure 10 times.

[2] Developer: 5.40 g metol, 6.60 g hydroquinone, 78.0 g crystalline Na_2SO_3 0.4 g KBr and 35.0 water-free Na_2CO_3 are dissolved in water and brought up to 1 litre. When used, the developer is diluted 1 : 2.

[3] Fixing bath: 2.5 kg $Na_2S_2O_3$ and 500 g $Na_2S_2O_5$ are dissolved in 10 litres of water

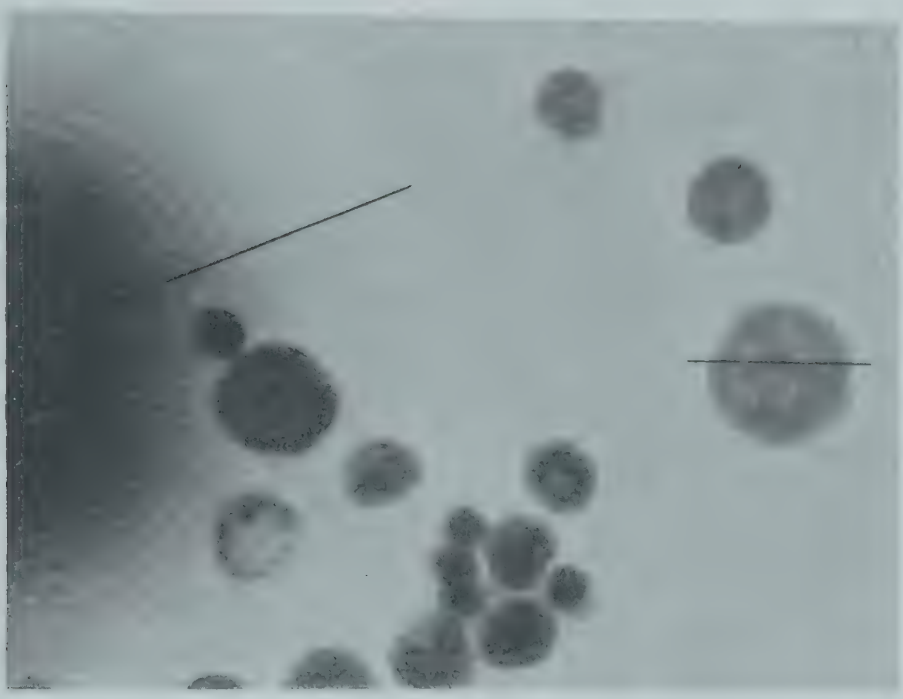


Fig. 14. Measurement plate with calibration system, obtained at 2750 Å. The photometry is performed along the indicated lines with a self-registering microphotometer.

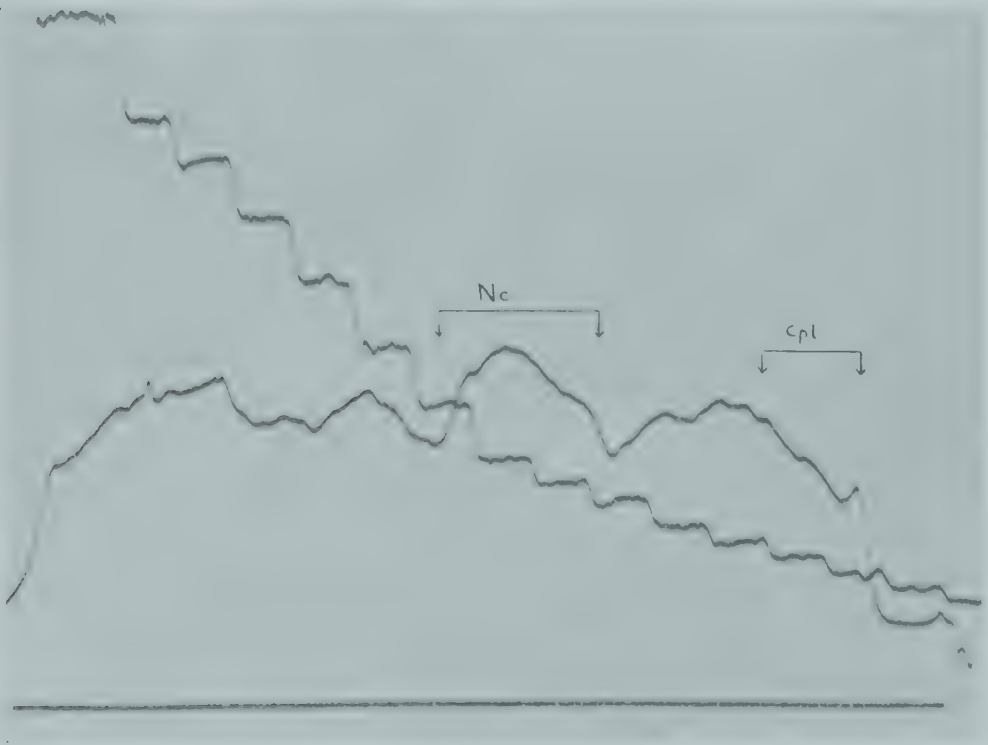
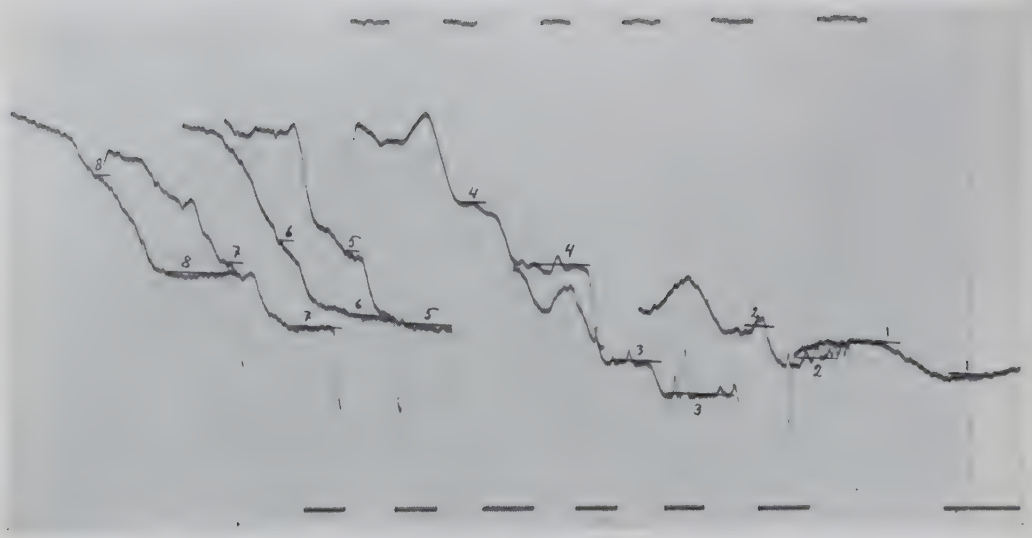
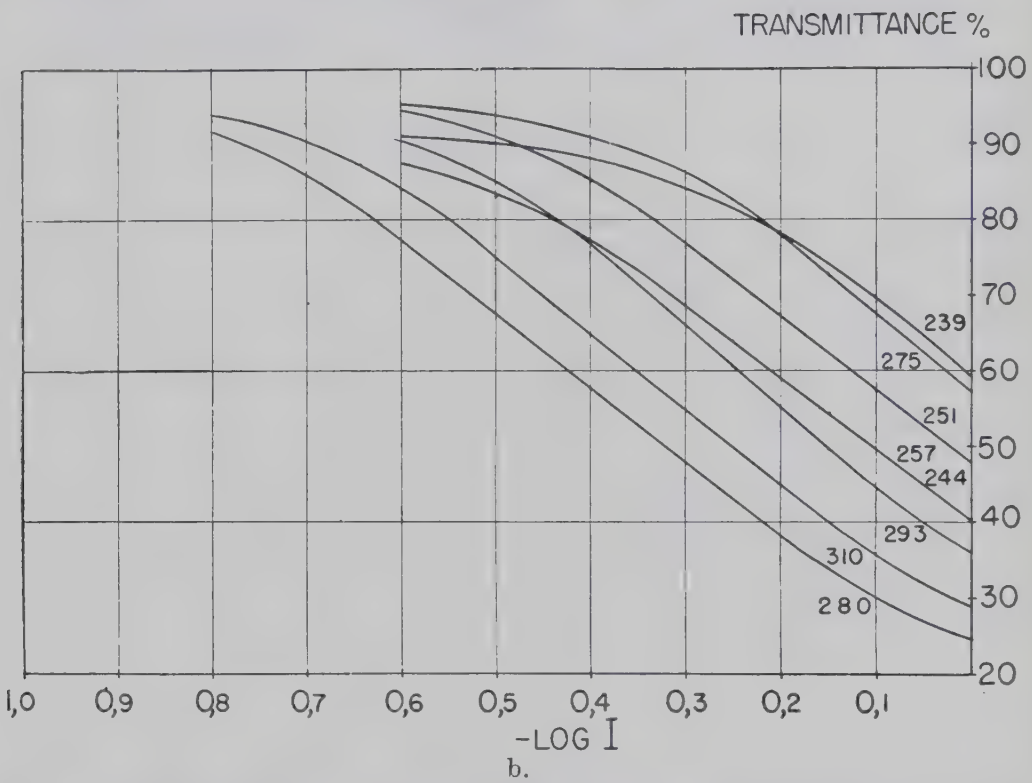


Fig. 15. Microphotometer records from the plate in fig. 14. Nc indicate the range of the nucleolus and cpl the range of the cytoplasm to the right of the nucleus. From the record of the sector steps (with known absorption values) and the record of the cell, the absorption of each point in the cell along the photometered line can be calculated.



a.



b.

Fig. 16. a) Microphotometer records of the cytoplasm of the cell in fig. 14 from 8 different plates taken at the wavelengths Mg 310, 293 and 280 mμ (1, 2, 3), Cd 275 and 257 mμ (4, 5), Ni 251, 244 and 239 mμ (6, 7, 8). The numbered straight lines indicate the transmittances of the cytoplasm, the range of which has been obtained by making measurements on the corresponding plates. b) Graphical representation of the transmittances of the sector steps photographed simultaneously on the plates recorded in fig. 16 a.

and temperature as above. After fixing and washing the plates are air-dried. Fig. 14 shows a typical photograph of bone marrow cells and sector image at 2750 Å.

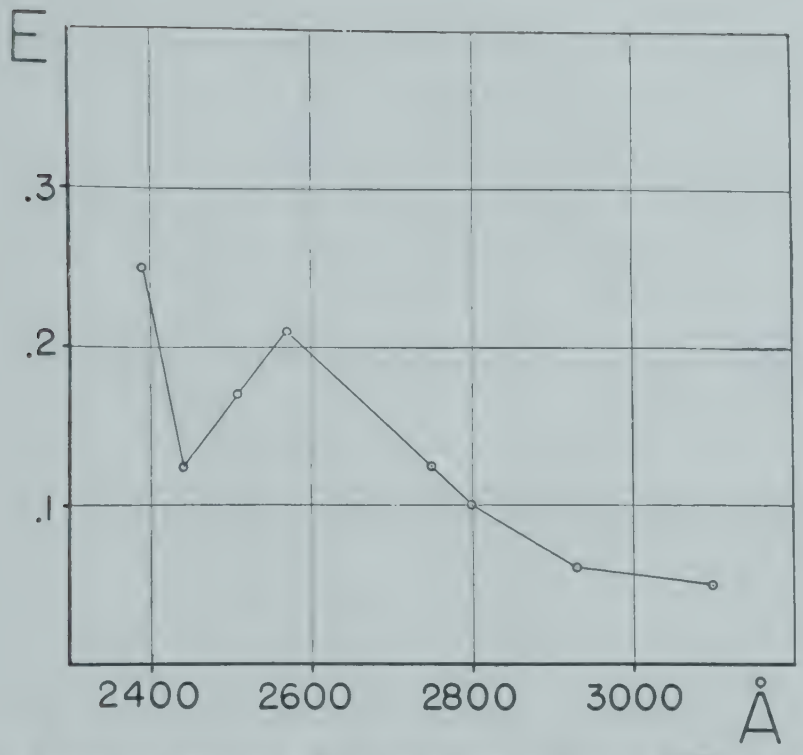


Fig. 17. Absorption spectrum of the cytoplasm of the cell in fig. 14 calculated from the records in fig. 16 a and the curves in fig. 16 b.

For determinations of the density a self-registering microphotometer of Siegbahn's construction with a thermoelement and Moll's microgalvanometer is used. The source of light is a tungsten lamp fed from an accumulator battery. The voltage (6.0 volt) is controlled by a precision voltmeter. The measurement surface of the plate can be reduced to 0.2×0.2 mm, by means of slits for the aperture of the thermoelement. This surface is adequate in view of the resolving power and enlargement obtained by the microscope optics. Ilford recording paper is used for registration of the galvanometer deflections. Fig. 15 shows a typical registration of the density of the cell and sector images. As the weakening of the light by the sector steps is known, the light absorption of the cell structures can be graphically calculated from the photometer curve with due consideration to what has been ascertained above (p. 26) as to the intensity distribution of the cell image.

Fig. 16 shows examples of a registration from the images of the same cell in the 8 wave lengths employed. For calculations of the absorptions in the structures of the cell the density steps of the corresponding sector images are illustrated graphically.

The light absorptions for a point in the cytoplasm are calculated and plotted to the absorption spectrum in fig. 17. From this curve

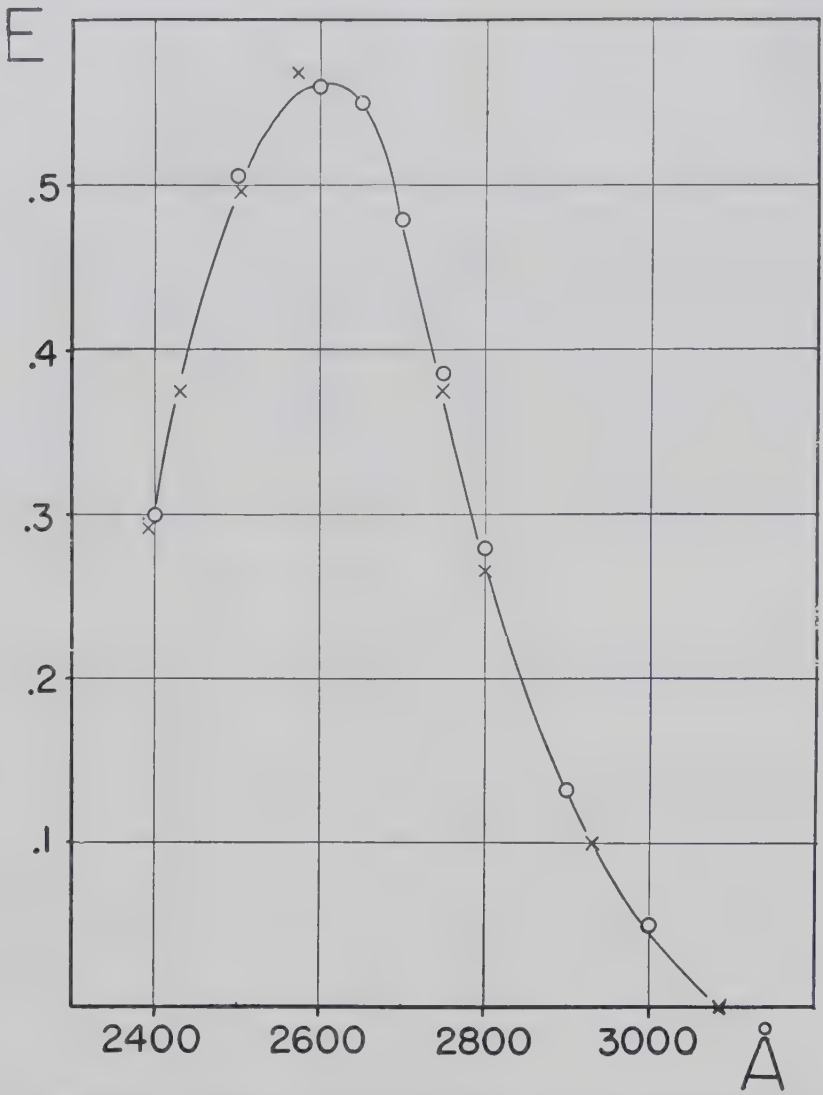


Fig. 18. Absorption spectrum of a 2.25 % ribose nucleic acid solution obtained 1) in a cellophane bag cuvette with the microspectrographical arrangement described in text (crosses) and 2) in a 10 μ Scheibe cuvette with a photoelectrical absorption spectrograph according to Warburg-Negelein (circles).

the composition of the protein and nucleic acid can be calculated. The basis for this calculation are described in Chap. 4, p. 47.

e. *Testing with pure substances.*

The above-described ultraviolet microspectrographic device was tested with known solutions in the following manner: A 2.25 % solution of protein-free ribose nucleic acid (prepared according to HAMMARSTEN 1947) was pipetted into a thin-walled, entirely ultraviolet-transparent (> 2000 Å) cellophan sac. A similar one was filled with aq. dest. The two sacs were placed edge to edge between the object glass and the cover glass of molten

quartz and pressed together. The surrounding edges were made air-tight with paraffin wax. The layer thickness was measured according to the procedure described above (table 1), after which the dividing wall between the ribose nucleic acid solution and the water was adjusted sharply in the plane of the object to the ultraviolet microscope. The absorption values were measured with the procedure described from 3100 Å to 2390 Å. The same 2.25 % solution was then measured in a 10 μ Scheibe cuvette with a photo-electric absorption measurement device according to Warburg-Negelein. For results see fig. 18, which shows an agreement with c. 5 % error in the individual measurement value.

In connection with these experiments investigations were also made to determine whether the light absorptions of ribose and ribodesose nucleic acids follow Lambert-Beer's law within a concentration range of 2.5 % > c. > 0.005 %. This is a matter of importance since the absorption constants found macrochemically can be applied in the case of cytochemical analyses. In this connection the light absorption was measured in 2.5 % solutions with 10 μ Scheibe cuvettes and the same solutions diluted to 0.005 % in 3.172 mm layers. The measuring was effected in a Warburg-Negelein device for photo-electric absorption measurement. The values are given in table 4 and show satisfactory correlation between the absorption constants obtained.

Table 4.

The extinction (E) and absorption constant (k) of ribose- and ribodesose nucleic acid at concentrations of 0.005 % to 2.5 %. Preparation according to HAMMARSTEN 1947.

Ribodesose nucleic acid				Ribose nucleic acid			
conc. %	layer thickness mm	E	k	conc. %	layer thickness mm	E	k
2	0.01	0.373	1.86	2.5	0.01	0.550	2.20
0.01	3.17	0.600	1.89	0.005	3.17	0.340	2.15

f. *Testing on extremely small objects*

Finally, the method was tested on extremely small objects of well defined and homogeneous biological material: individual bacteria in growth and resting phase respectively. From the former might be expected a nucleic acid spectrum (max. 2600 Å),

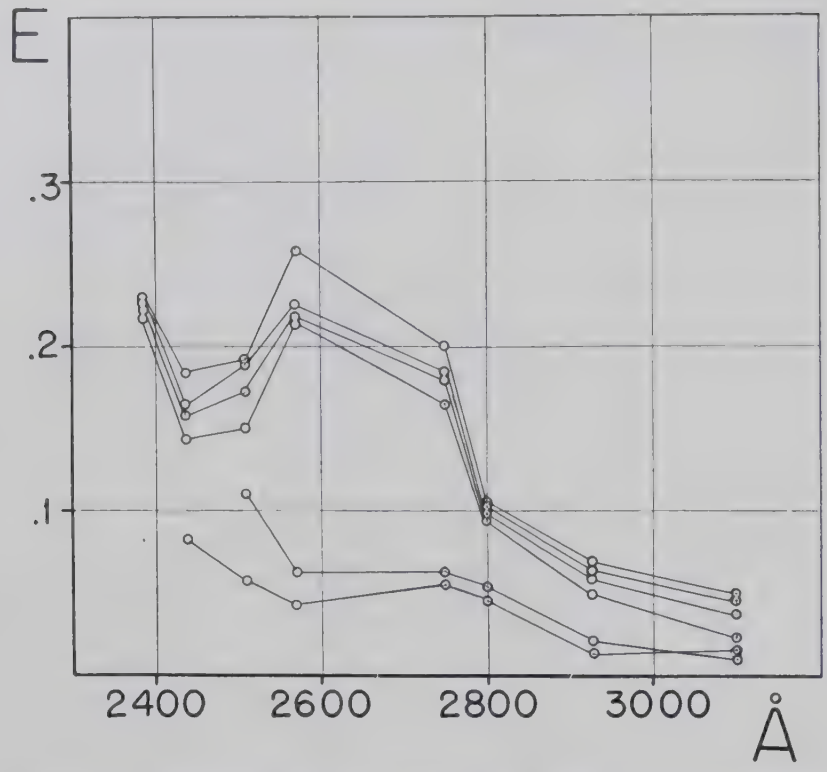


Fig. 19. Absorption spectra of single bacteria (B. cereus) obtained with the microspectrograph described. The upper four curves are from rapidly growing bacteria, the lower curves from bacteria in the resting phase.

from the latter a purely protein band (2800 Å) (see CASPERSSON, MALMGREN and THORELL, 1945, MALMGREN and HEDÉN, 1947). The results in fig. 19 show that this is the case and that the analyses from different bacteria in the same phase agree.

II. Measurement Apparatus for Microspectrography in the Visible Spectral Range.

The Soret band is most suitable for the determination of hemoglobin in bone marrow cells.[1] Here, too, owing to the steep slope of the absorption curve pure monochromatic light is necessary. Fig. 20 shows the absorption curve for oxy-hemoglobin and the extinction of different concentrations of oxy-hemoglobin in 1 μ layer in a number of the mercury emission lines.

Measuring device (fig. 21). The source of light A is a water-cooled Philips high pressure mercury lamp fed with stabilized alternating current 600 volts 50 per. The light is dispersed by a Zeiss prism monochromator with 90° constant deflection angle (B). The height

[1] For a review concerning absorption spectrography of hemoglobin and related compounds, see HOLDEN 1945.

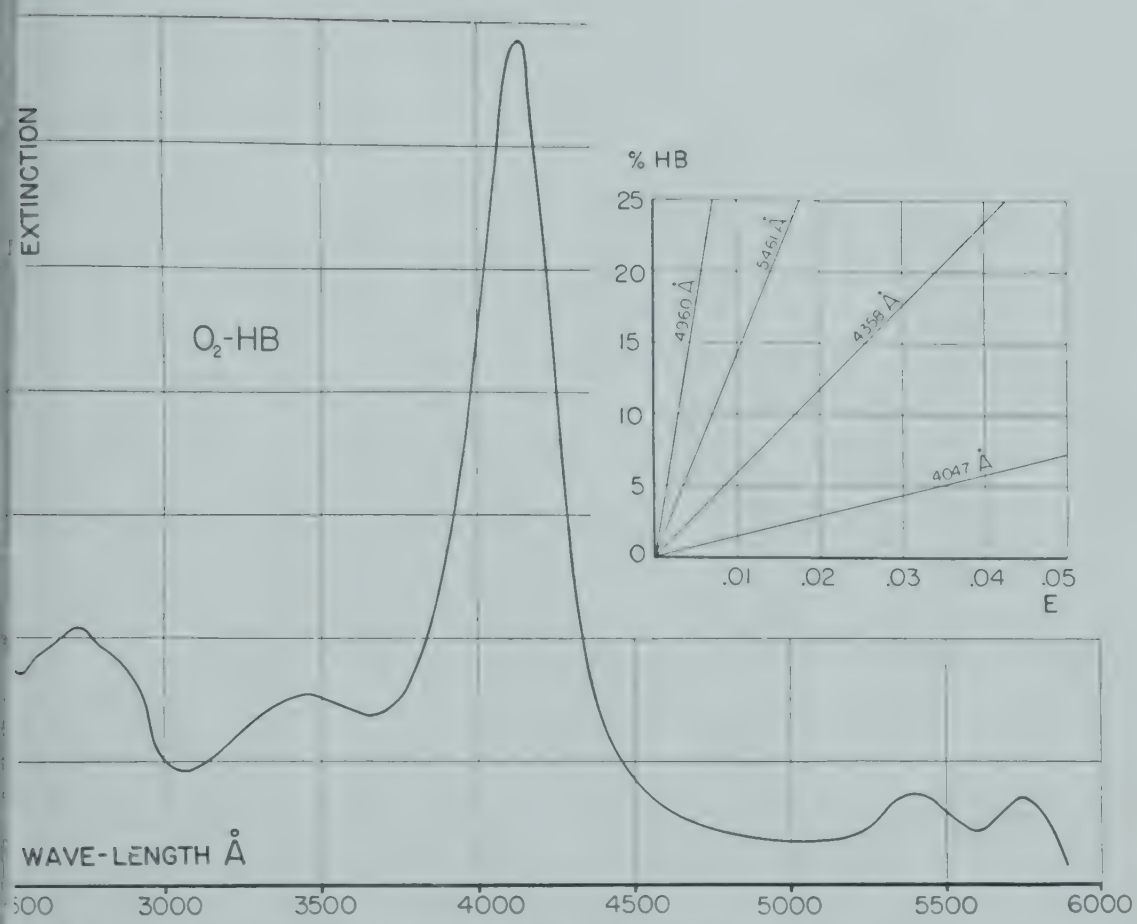


Fig. 20. Absorption spectrum of a 0.2 ‰ oxy-hemoglobin solution, measured with a Beckman quartz spectrophotometer. The smaller figure shows the extinctions of different concentrations of oxy-hemoglobin in 1μ layer at the mercury emission lines.

of the prism is 50 mm. and the dispersion at 4500 Å is 78 Å/mm. Stray light is filtered off with the Schott filter combination D. The optical path is so arranged that the monochromator exit slit (C) functions as an illumination field slit and the microscope condenser diaphragm (E) as a pure aperture diaphragm. This makes illumination with a known aperture possible at the same time as a highest possible spectral purity.

As the measurement objective (F) is used an apochromatic 90 × objective lens with numerical aperture 1.30 and 2.8 mm focal distance. The ocular is a compensating ocular 10 ×.

From the microscope optics the image is thrown by a 90° prism (G) towards the photo-cell aperture (I). The prism-holder is mechanically very firm, and it can be moved with two precision micrometer screws round a horizontal and a vertical axis. The image is

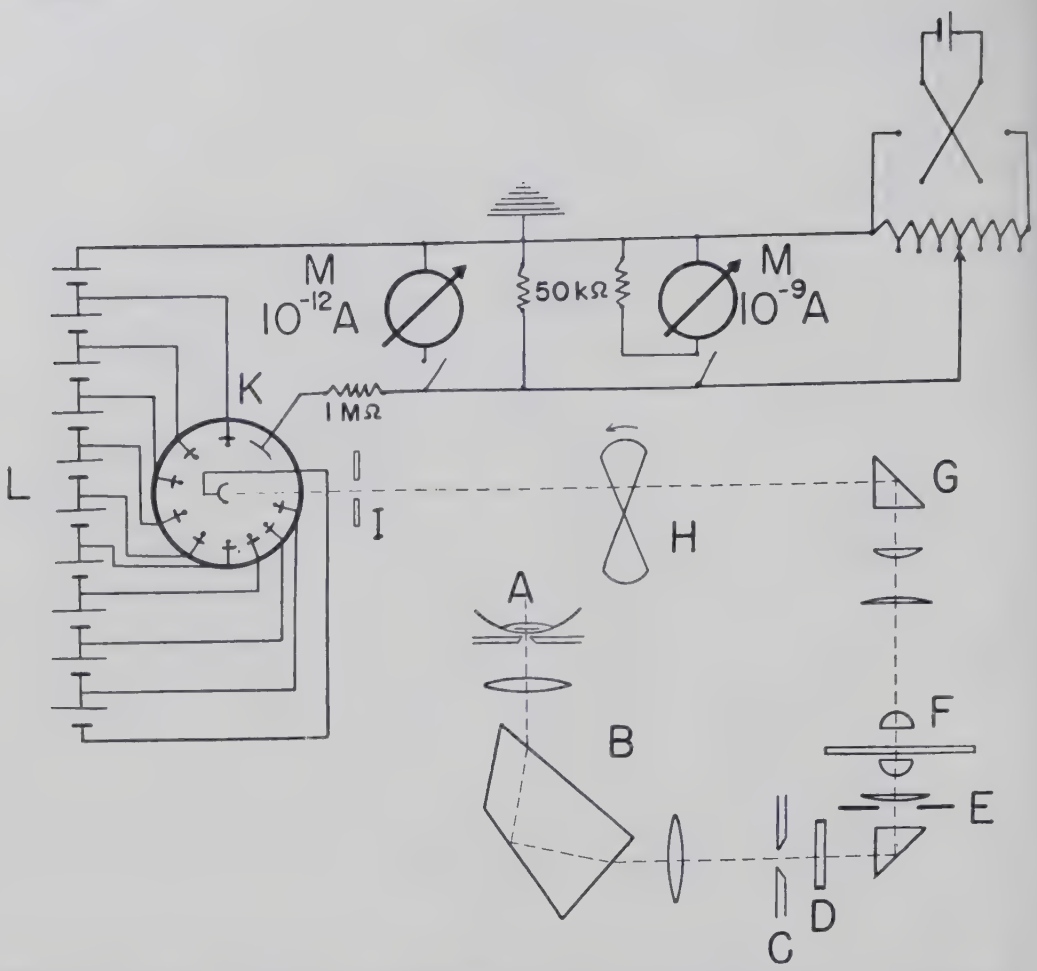


Fig. 21. Scheme of the microspectrograph for the determination of hemoglobin in single cells. A light source, B monochromator, C monochromator exit slit, D filter combination, E condenser diaphragm, F objective lens, G movable prism, H rotating sector, I photo tube diaphragm, K electron multiplier photo tube, L batteries feeding the electron multiplier tube, M high current sensitive galvanometer or mirror reflex galvanometer with lower sensitivity. For further explanations, see text.

thereby moved over the photo-cell aperture, whereby steps cut in the sockets of the micrometer screws render possible measurements of definite points in the cell image and of the intensity of the incident unweakened light in the immediate neighbourhood of the cell. This arrangement calls for extremely even illumination of the object, which is secured satisfactorily with the monochromator employed in the level of altitude of the mercury lines.

For calibration of the photocurrent a rotating sector (H) is inserted between the prism and the photocell aperture, divided into steps which can be successively introduced into the light path.

The distance from the prism to the photocell aperture is so chosen that the photocell aperture corresponds to an object surface with a diameter of 0.2 μ. The photocell K is an RCA's 931-A with 9

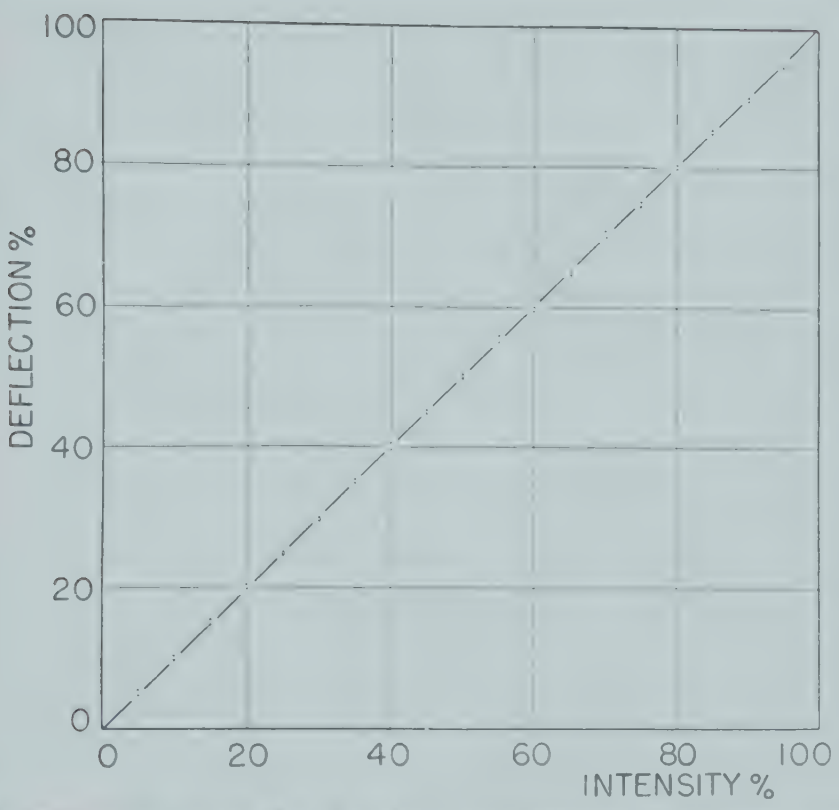


Fig. 22. Calibration curve for the photo tube system, illuminated with light intensities in the order of magnitude of 10^{-15} watt/cm². The galvanometer deflection in % (full scale 500 mm) as a function of the intensity of the incident light against the photo tube.

steps electron multiplier amplification. The potentials of the steps are obtained from the series-coupled batteries L. The measuring instrument is a Moll's galvanometer with high current sensitivity (M). The coupling is shown by the figure. The whole light measurement device is shielded with 1 mm copper sheeting. With the light intensities employed in this investigation (c. 10^{-15} watt/cm²) the readily measurable current strengths in the order of magnitude 10^{-10} amp. are obtained. The deflections are strictly linear against the light intensity with the total light strengths of this magnitude (see fig. 22). The stability of the photocell system proves to be fully sufficient for the purpose in this case, the variations do not usually exceed $\pm$ 0.005 of the intensity of incident light.

Fig. 23 shows measurement results from an "orthochromatic" erythroblast. With known layer thicknesses the hemoglobin concentration at individual points can be calculated from the curve, attention being paid to what has been ascertained above concerning the optical conditions in the cell. As the light absorption of these cells in 4047 and 4358 Å is due practically

exclusively to hemoglobin, these measurement values also permit of calculations of the total amount of hemoglobin in the cell. This "total extinction" can be calculated as the product of the mean extinction and the surface of the cell object measured on a photographic plate. In the case of these symmetrically built precursors of red blood cells and when knowing the shape of the cell (se above table 1) the total extinction also can be calculated by means of graphical integration from the "extinction curve" as its rotation volume.

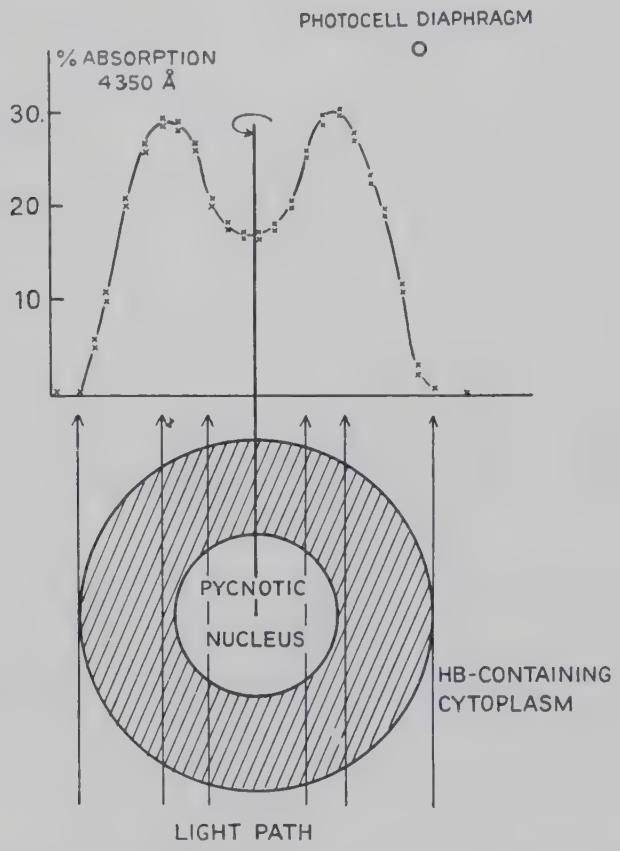


Fig. 23. The principle for hemoglobin determination in a single cell (specifically, determination of the prosthetic group). The curve shows measurements on a living "orthochromatic" erythroblast in physiological saline solution. In order to keep the hemoglobin in its oxy-form, oxygen-bubbles are present between the object slide and the cover glass. The values in the curve are obtained by moving the image of the cell with the microspectrograph prism G (fig. 21) along the cell diameter over the photo tube diaphragm I. By knowing the shape and dimensions of the cell (see table 1 with text), the hemoglobin concentration in each point along the diameter can be calculated. Unspecific losses of light must be considered, (see fig. 37). By integrating the measurement curve over the cellular volume, as indicated by the arrow, the total amount of hemoglobin in the cell can be calculated.

CHAPTER 4.

Cytochemical Analyses of Normal Bone Marrow.

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A. Material and Technique.

1. Material.

Experimental animals were chosen which have the same type of blood cell renewal as man. The main part of bone marrow cells for the investigation were obtained from c. 500 healthy adult rats (250—300 g), and about 30 rabbits (2—4 kg). The blood values of the animals were taken for control purposes and compared with the normal values indicated by KLIENEBERGER-CARL (1927) and STASNEY and HIGGINS (1935). In all the cases bone marrow from the femur was employed.

The normal material from homo comprised 22 healthy persons aged 20—30 years of age. The bone marrow was obtained by means of sternal puncture according to NORDENSON. (NORDENSON, 1935.) The puncture was taken in corpus sterni. In all the cases the number of red and white blood cells were counted, and the hemoglobin content in the blood controlled. Subjectively the experimental persons felt well at the time of the puncture, and there was nothing of note in the anamnesis. (Age and sex do not affect the composition of the bone marrow. (SEGERDAHL, 1935), nor does the place from which the bone marrow punctate is taken seem to play a significant role (NORDENSON, 1935, STASNEY and HIGGINS, 1939).

The preparations of the cells were made in the following manner: A piece of bone marrow was taken from the femur or from the sternal puncture respectively and broken up with two needles in a drop of physiological saline solution on a well-cleaned quartz slide. Preferably the saline solution indicated by Tyrode was used. A cover glass was layed over the cell suspension, after which the edges were closed with vaseline to prevent drying.

A preparation made in this way will keep for several hours without observable changes. In exceptional cases, for instance when it is necessary for calibration purposes to have a more stable preparation, 0.1 g NaCl in Tyrode's solution can be replaced by 0.2 g $LaAc_3$, for the polynucleotides[1] are precipitated intracellularly (HAMMARSTEN and TEORELL, 1928).

Before an account is given of the results of the analyses, a summarized description will be given of the different points of the investigation.

2. Light Absorption Measurement.

The technique for measuring light absorption has been described above (Chap. 3). The absorption curves obtained in ultraviolet are dominated by two groups of substances, the polynucleotides and the protein substances.

The light absorption of the polynucleotides has a maximum at 2600 Å, which is caused by the pyrimidine ring in the nitrogen-containing basic component in the polynucleotides (see fig. 10, Chap. 3 and fig. 24). The light absorption is practically identical for the two kinds of polynucleotides. Mononucleotides and free

[1] In describing the results of the analyses on the cell material, instead of the term nucleic acid the more adequate name polynucleotides is used below.

purine and pyrimidine bases, which may give rise to the absorption band, are found in comparatively low concentrations in ordinary cell material (HAMMARSTEN, 1947).

The ultraviolet absorption of the protein substances is conditioned by their constituent amino acids. Tryptophan, tyrosine and phenylalanine have selective light absorption in that wavelength range. The absorption band at 2800 Å in the case of the majority of protein substances is conditioned by these amino acids (DHÉRÉ, 1909). The absorption maxima for tyrosine and trytophan is between 2750 Å and 2800 Å (HOLIDAY, 1936), that of phenylalanine 2600 Å (FERAUD et al. 1935), (see fig. 24). In comparison with the tryptophan and tyrosine band, the latter is of but little importance; the light absorptions of tryptophan: tyrosine: phenylalanine are about in the following ratios 1 : 0.5 : 0.05 (BECKER, 1935).

The other amino acids have no, or very little, selective absorption. Below 2500 Å the light absorption of proteins rises rapidly. All the amino acids contribute to this non-selective light absorption (fig. 24).

The light absorption constants of all the above-mentioned substances has been determined by means of experiments on pure substances (see ELLINGER 1937). Thus from the ultraviolet absorption spectrum the presence of polynucleotides, tyrosine

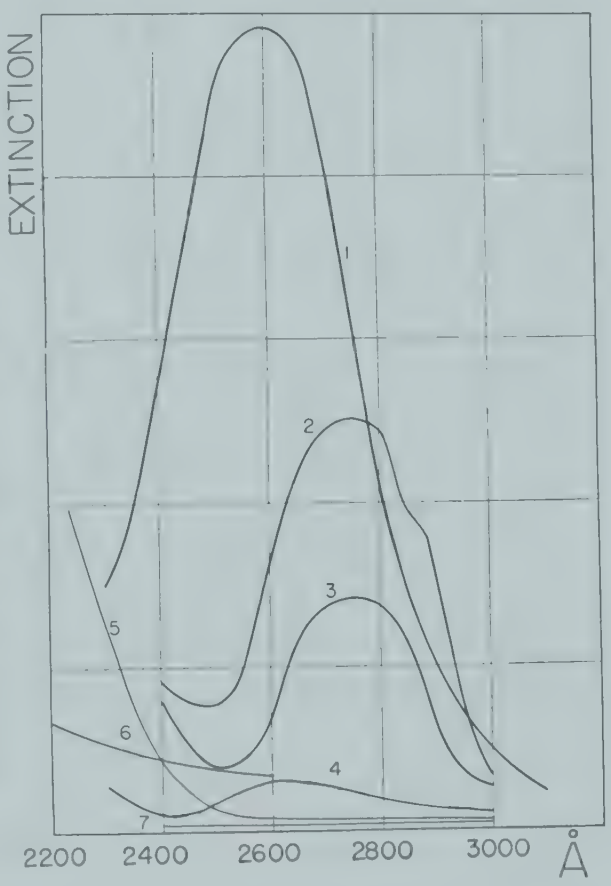

Fig. 24. Schematic representation of the main components of an ultraviolet absorption spectrum obtained from cytological material. 1. nucleic acid, 2. tryptophan, 3. tyrosine (acid solution), 4. phenylalanine, 5. other amino acids, 6. Raleighs extinction and 7. constant unspecific losses of light such as reflection and refraction. The relation between the concentrations of nucleic acid, tryptophan, tyrosine and phenylalanine is c:a 0.4 : 1 : 1 : 1. The other components are in arbitrary magnitudes.

and tryptophan can be demonstrated. This is done most simply by means of graphic dispersion of the absorption spectrum into its components.

As the thickness of the light absorbing layer is known (see below 5), the concentration of polynucleotides can also be calculated from the extinction in its absorption maximum at 2600 Å, and the concentration of tyrosine and tryptophan from the extinction in their maximum between 2750 and 2800 Å. In the presence of polynucleotides, however, the latter is connected with large errors, depending on the high polynucleotide absorption.

It is of interest to be able to estimate also the concentration of protein substances. If the contents of tyrosine and tryptophan in the protein substance are known, this can be done from the light absorption at 2750—2800 Å. For tissue protein in general the contents of tyrosine and tryptophan vary within somewhat narrow limits; tyrosine between 3 % and 6 %, with a mean value of 5 %, tryptophan between 1 % and 3 % with a mean value of 1.5 % (see BLOCK, 1938). If a "standard protein" is defined as containing 5 % tyrosine and 1.5 % tryptophan, 10^{-10} mg of "protein" per μ^2 in the absorption maximum between 2750 and 2800 Å can be calculated to give an extinction of 0.007. At 2570 Å the extinction will be 0.004. With these values, the protein content can be roughly estimated from the light absorption data.

A non-selective component in the light absorption spectrum is caused by unspecific light dispersion owing to inhomogeneities within the larger cell structures. These losses of light can be calculated roughly from empirically found values with a simplification of the formula given by RALEIGH:

$$E = k \cdot \frac{1}{\lambda^4}$$

where E = the extinction, k = a constant, λ = the wave length. In living cell material, where the protein substances are in a native state, however, the loss of light caused by Raleigh's extinction is comparatively small. Consideration need be paid to it only in exceptional cases.

Certain sterols occurring in the cell exhibit selective light absorption within the middle of ultraviolet and may give rise to light absorption in that wave length range. Their low concentrations in ordinary cell material, and the fact that their selective absorptions mainly lie at shorter wave lengths (2300—2400 Å) than

those concerned here, make this less probable. A simple experiment confirms this: Smears of bone marrow on quartz glass were fixed with formalin vapour. The light absorption at 2570 Å was determined photographically of the cells. Afterwards lipoids were extracted from the preparation (according to Bloor). By means of Maltwood's microsystem of coordinates, the light absorption at 2570 Å was again determined. For the values see table 5. No differences, apart from experimental errors, can be observed.

Table 5.

The extinctions at 257 mμ of cytoplasm and nucleus in bone marrow cells before and after lipoid-extraction.

Cell	E_{257} before	E_{257} after	$\left(\frac{E_1-E_2}{E_1}\right)$ %
$1_{nucleus}$	0.270	0.265	+ 1.8
$1_{cytoplasm}$	0.155	0.155	0
2_n	0.355	0.360	— 1.4
2_c	0.155	0.160	— 1.4
3_n	0.350	0.357	— 2
3_c	0.285	0.280	+ 1.7
4_n	0.260	0.264	— 1.5
4_c	0.142	0.140	+ 1.4
5_n	0.470	0.475	— 1.1
5_c	0.086	0.090	— 4.6
6_n	0.520	0.505	+ 2.9
6_c	0.232	0.230	+ 0.9

The determination of a complete absorption spectrum as outlined above (Chap. 3) is comparatively time-consuming. As bone marrow cells of the same type have a very homogeneous appearance in ultraviolet (see below), it is justifiable to limit this analysis material to a certain extent. As a rule, therefore, about 10 complete absorption curves have been taken of each type of cell, *i. e.* the material has been analysed till definitely constant results were reached.

The relation between polynucleotides and proteins can also be calculated from the relation between the extinctions at 2570 and 2750 Å, after possible corrections have been made for unspecific losses of light. In order to be able to analyse larger material, a number of determinations of the light absorption at only 2570 Å and 2.750 Å has been made. This implies a considerable saving of time since *inter alia*, the light can be obtained with the same

electrode material (cadmium). From the quotient between these light absorption values the relation between polynucleotides and protein has been calculated according to the following:

For 1 cm of a 1 % polynucleotide solution the decadal extinction coefficient is 21 at 2570 Å and 16 at 2750 Å. The corresponding

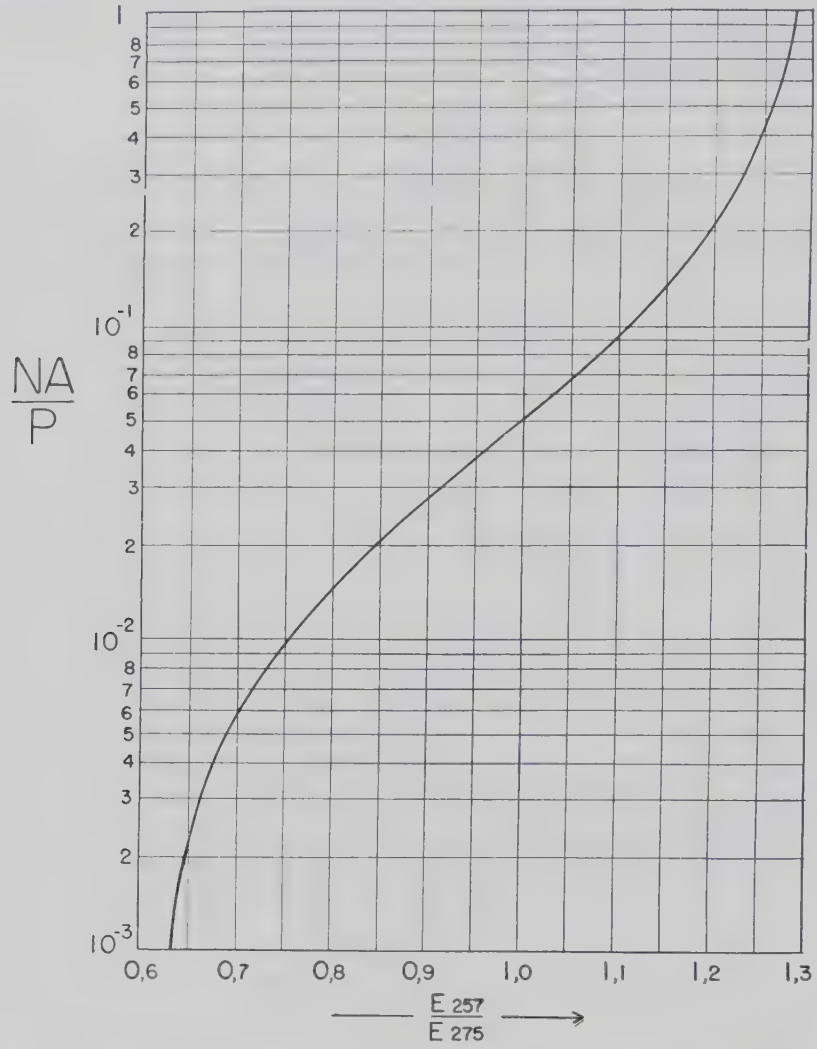

Fig. 25. The relation between the concentration of nucleic acids (NA) and the concentration of proteins (P) as a function of the ratio between the extinctions at 257 mμ and 275 mμ. For details, see text.

values for a 1 % protein solution according to the above are 0.40 and 0.70. In a mixture of the polynucleotide concentration (NA) and the protein concentration (P) the relation between the extinction coefficients at 2570 Å and 2750 Å is:

$$Q = \frac{21 \cdot (NA) + 0.40 \cdot (P)}{16 \cdot (NA) + 0.70 \cdot (P)}; \quad \frac{(NA)}{(P)} = \frac{0.70 \cdot Q - 0.40}{21 - 16 \cdot Q}$$

The graphic expression for this equation is shown in fig. 25, from which the relation NA/P can be obtained direct from a certain quotient value. The magnitude of the material analysed in this way appears from the tables below.

Calculation of the concentration and amount of hemoglobin from light absorption data in the visible spectral range has already been described in Chap. 3, p. 43.

3. Ultraviolet Microphotography.

For taking microphotographs in ultraviolet a device constructed by A. KÖHLER, 1933, was used.

As is shown by figs. 10 and 24, the light absorption at 2600 Å of polynucleotides is very great in comparison with that of the protein substances. In a microphotograph taken at that wave length under suitable conditions the cell structures which contain polynucleotides stand out specially distinctly, owing to their high light absorption. The following rough calculation makes this clear: A 5 μ thick layer with an extinction at 2600 Å of 0.2 can be calculated to contain 2 % of polynucleotides. If the same layer contains only protein substances, the latter must — to yield the same light absorption at 2600 Å — have the concentration of 100%. This is impossible. Thus a high light absorption at 2600 Å, corresponding to the "dark" areas of the ultraviolet microphotograph, cannot reasonably have been caused by protein substances alone, but as a rule shows the polynucleotide component. It should be emphasized, however, that consideration must be paid to the unspecific losses of light, such as reflection, refraction and light dispersion, and to the technical conditions under which the microphotograph was taken (see Chap. 3). The presence of polynucleotides cannot therefore be considered demonstrated solely by a high absorption on the ultraviolet microphotogr ph. Only a complete absorption spectrum of the part of the cell in question can be decisive.

Thus over and above a picture of the cytology of the intact living cell, ultraviolet microphotography of the different forms of blood cells can also afford a conception of the polynucleotide distr bution in the cell. As an important step in the investigation the cytological picture of every myeloid cell type at 2570 Å, *i. e.* very near the polynucleotide absorption maximum, will be described below. Unless otherwise stated under the respective pictures, for the objective lens a quartz monochromat 1.6 mm was used, an aperture for

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incident light of 2·30°, ocular 5×, tube length 160 mm, and a distance from the object to the plane of the image 57 cm. Agfa Platten B was used throughout. The microphotographs were taken with a constant exposure time (4 sec.) and were developed and copied under as constant conditions as were practically possible.

500 ultraviolet microphotographs of normal bone marrow from the above-mentioned material were taken in this way. As each plate contains an average of 30 cells, the following description is based on the examination of about 20,000 normal living bone marrow cells.

4. Localization of Ribodesose Polynucleotides.

Ribodesose polynucleotides can be cytologically identified with a microchemical reaction, the so-called Feulgen nucleal reaction. This is based on the capacity of fuchsin sulphuric acid to develop a red colour with the aldehyde group in the pentose which is set free from the ribodesose polynucleotides after acid hydrolysis. In cytological material the reaction can be considered specific for ribodesose polynucleotides. (Feulgen and Rossenbeck, 1924.) The specificity of the method has been questioned by certain authors, but no sound experimental reasons have been advanced.

The method does not permit of any quantitative determinations, but to distinguish between ribodesose and ribose polynucleotides, it is a valuable supplement to the microspectrographical analyses.

The cell preparations for the Feulgen reaction were bone marrow smears which, while still moist, were fixed for a minute in formalin vapour, which does not affect the Feulgen reaction under the experimental conditions. Compared with living cells, such preparations exhibited very small changes in the cytological picture. After hydrolysis for 4 min. in 1n HCl at 60° and rinsing in water, lipoids were extracted from the preparation. This procedure will exclude some other substances occurring in cytological material; Feulgen's plasmal, plasmalogen and other lipoid aldehydes, which have free aldehyde groups after hydrolysis. Subsequently they were allowed to develop colour with fuchsin sulphuric acid, prepared according to Honneth (1940).

5. Cytometry.

Certain discussions in the following chapters will also be based on quantitative cytological data. In this connection the dimensions of the cells and certain parts of the cells were found as follows:

With the procedure described above in table 1 (p. 20, Chap. 3) it was shown that, with the preparation technique employed the living bone marrow cells could be considered to be approximately spherical. Thus the cell volume is obtained from the diameter, which is measured on the ultraviolet photograph or in phase contrast microscope with an ocular micrometer. The thickness of the light-absorbing layer for a certain measurement point can also be calculated from the diameter.

The size of the nucleolus was measured on smear preparations stained with Feulgen's nuclear reaction, as the nucleolus-associated chromatin could be studied on them at the same time. To facilitate cell classification, the preparation was after-stained slightly with May-Grünwald's methylene-blue eosin solution. The measuring was effected at 1,500 times linear magnification with a Zeiss ocular screw micrometer. This arrangement permits of an accuracy in measuring down to 0.2 μ, *i. e.* to the maximum resolving power of the microscope in visible light.

The diameter values cannot be transferred to nucleolus volume, as the degree of cell flattening in the smear preparations cannot be considered to be the same for all cells. Nor, as the nucleolar mass in a nucleus is often distributed over several nucleoli, is the sum of the diameters of the nucleoli suitable as a measure. The best thing is to calculate from the diameter values the total optical cross-section area of the nucleolar mass. The approximations which are given here are of slight importance compared with the great changes in the nucleolar dimensions during the course of blood cell renewal (see below).

B. The Analysis Results.

In the following account of the analysis data the classical division and nomenclature for the cellular development phases which was described in Chap. 1 (p. 6) is employed. Hematopoiesis is, however, a continuous cellphysiological process. A distinction into different types cannot be strictly made, but the border-lines are more or less fluid.

1. Granulocytopoiesis.

a) *The myeloblast* (figs. 26 a, 27 a, 28, 29, tables 6 and 7).

1. A spherical cell with a mean diameter of 13 μ (table 6). The cytoplasm, which forms a relatively thin layer round the nucleus,

contains considerable amounts of at 2570 Å strongly absorbing substances. These are usually uniformly distributed in the cytoplasm, but in some cells small vacuoles can be observed.

The nucleus is large and delimited by a distinct nuclear membrane. In the karyoplasm, apart from a few small absorbing chromatin granula, two to four large, strongly light-absorbing nucleoli can be discerned at 2570 Å. The mean area of these in smear preparations is 39 μ^2 (table 6).

Table 6.

Cytometry of the bone marrow cells from rat and human (samples from 10 individuals).[1]

	Cell diameter μ	Area of nucleolus in μ^2
Myeloblast	13.3 ± 0.1 $\sigma = 0.8$	38.8 ± 0.1 $\sigma = 0.5$
Promyelocyte	16.3 ± 0.13 $\sigma = 1.02$	12.7 ± 0.2 $\sigma = 1.0$
Myelocyte	12.9 ± 0.15 $\sigma = 1.37$	5.1 ± 0.2 $\sigma = 1.0$
Mature cell	10.2 ± 0.14 $\sigma = 0.8$	—

2. Absorption curves from points in the cytoplasm show a prominent maximum at 2600 Å (fig. 28). The same courses have the light absorption of the nucleoli. The mean value of the quotients between the light absorptions of the cytoplasm at 2570 Å and 2750 Å is at 1.26 (table 7).

3. With the Feulgen nucleal reaction only the fine chromatin structures develop colour. The cytoplasm and nucleoli, which both had a light absorption of a polynucleotide character, are entirely Feulgen-negative. The site of the nucleolus can be discerned, as

---

[1] Any significant differences in cell sizes between cells from human and rat cannot be detected.

For the statistical treatment of the measurements the following methods have been adopted:

The dispersion is calculated in accordance with the formula $\sigma = \sqrt{\frac{\Sigma\, a^2}{n-1}}$, where a is the deviation from the mean value and n the number of observations.

The mean error of the arithmetical mean is calculated in accordance with the formula $e(M) = \pm \frac{\sigma}{\sqrt{n}}$.

the thin, strongly disrupted superficial layer of nucleolus-associated chromatin stands out clearly (fig. 27 a).

Table 7.

The quotient $\frac{E_{257}}{E_{275}}$ *for points in the cytoplasm of myeloblasts.*

Cell	E_{257}	E_{275}	Q
1	0.640	0.485	1.32
2	0.600	0.480	1.25
3	0.670	0.540	1.24
4	0.500	0.395	1.27
5	0.685	0.545	1.25
6	0.775	0.590	1.31
7	0.590	0.490	1.20
8	0.670	0.550	1.22
9	0.265	0.210	1.26
10	0.580	0.470	1.24
11	0.765	0.600	1.27
12	0.630	0.490	1.29
13	0.600	0.500	1.20
14	0.810	0.620	1.30
15	0.590	0.475	1.24

$M = 1.26 \pm 0.01$

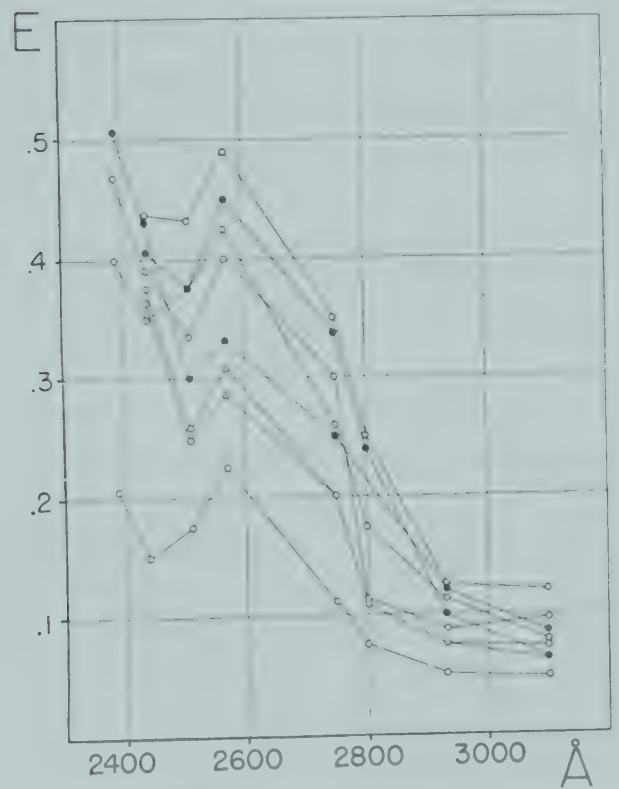


Fig. 28. Absorption spectra of points in the cytoplasm (empty circles) and in the nucleolus (filled circles) of myeloblasts.

All the data show that the cytochemical picture of the myeloblast is dominated by high concentrations of ribose polynucleotides in cytoplasm and nucleoli. From the data of the light absorption the concentration can be calculated to be about 5 %. The ratio of nucleic acids to protein with an average tyrosine-tryptophan content is calculated from the quotients $\frac{E_{257}}{E_{275}}$ as 1 : 3 on an average (fig. 29).

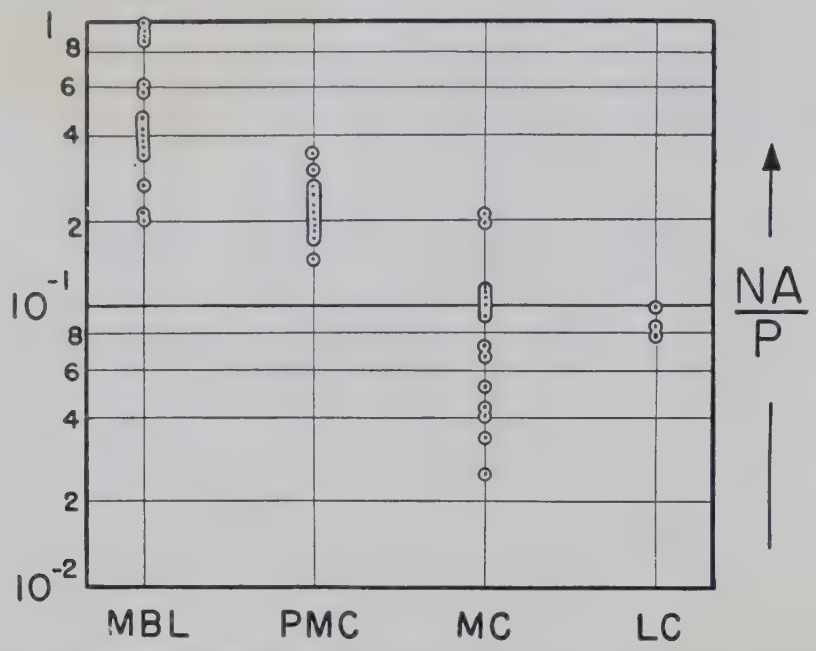


Fig. 29. The relation between the concentration of ribose polynucleotides (NA) and the concentration of protein (P) in the cytoplasm during granulocytopoiesis. The points are calculated from the quotient $\frac{E_{257}}{E_{275}}$ with the use of the diagram in fig. 25. Mbl = myeloblast, Pmc = promyelocyte, Mc = myelocyte and Lc = mature granulocyte.

b) *The promyelocyte* (figs. 26 b, 27 b, 29, 30, tables 6, 8).

1. A round cell with a diameter of 16 μ (table 6). The cytoplasm is large and contains moderate amounts of absorbing substances. In the latter are to be seen considerable lighter areas, in certain cells slightly granulated. In all probability these are the sites of the commencing formation of granula.

The nucleus is round, somewhat smaller than that of the preceding cell and delimited by a distinct membrane. The ultraviolet-absorbing chromatin is more or less collected in smallish aggregations, generally situated close to the nuclear membrane. In addition the nucleus contains 1—3 moderately large, strongly light-absorbing nuclear bodies (fig. 26 b).

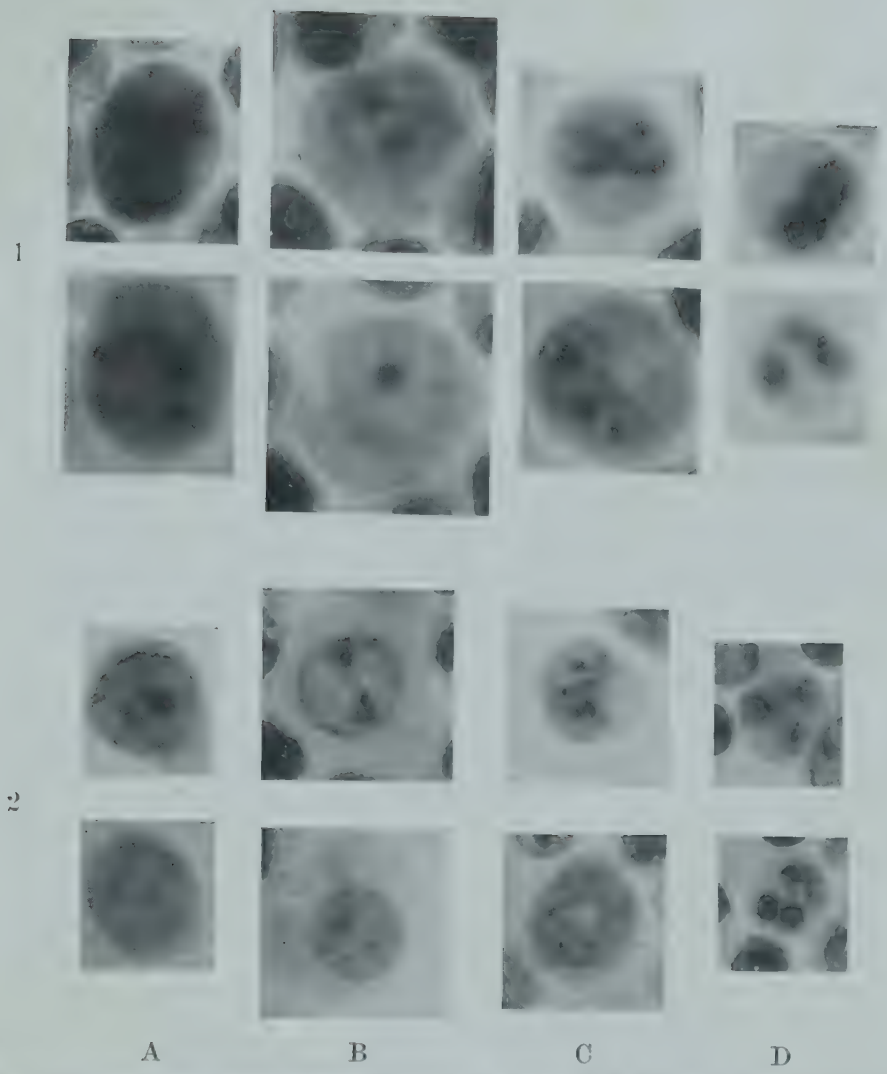


Fig. 26. Ultraviolet microphotographs taken at 2570 A of living granulocytopoietic cells in physiological saline solution. Row 1: human cells obtained by sternal puncture (1,500 × enlargement). Row 2: bone marrow cells from rat (1,150 × enlargement). A myeloblasts, B promyelocytes, C myelocytes and D granulocytes. For technical data, see text.

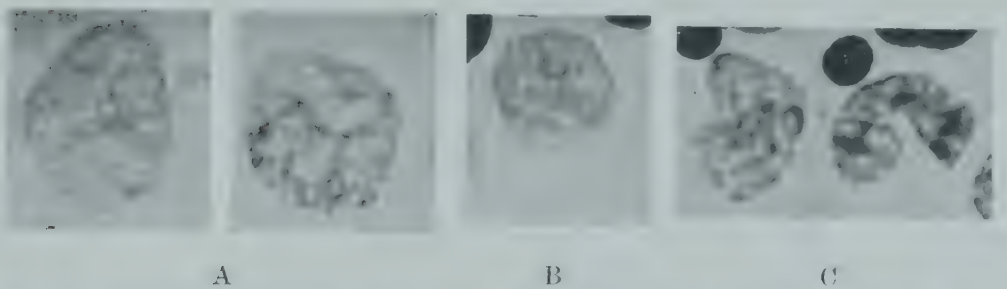


Fig. 27. Granulocytopoietic cells stained by the Feulgen reaction. A myeloblasts, B promyelocyte, C myelocyte and a mature granulocyte. Note the disrupted nucleolus-associated chromatin in the earlier development stages (A, B) and the Feulgen-positive chromocentra in the mature cell.

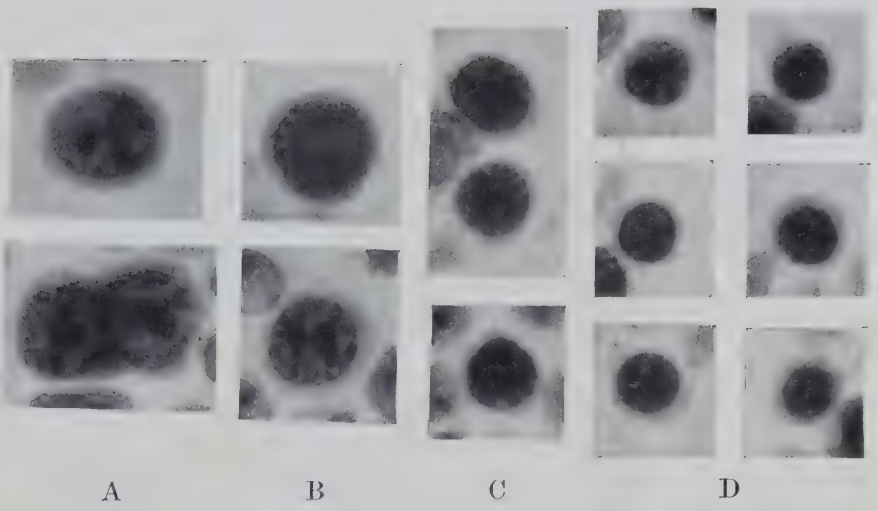


Fig. 34. Ultraviolet microphotographs taken at 2570 Å of erythropoietic cells. A proerythroblasts, B basophilic erythroblasts, C polychromatic erythroblasts and D orthochromatic erythroblasts. Magnification 1150 ×.

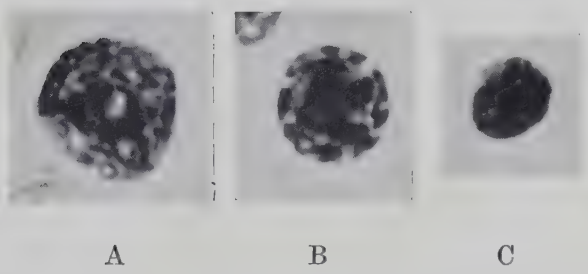


Fig. 35. Erythropoietic cells stained with the Feulgen reaction. A proerythroblast, B basophilic-polychromatic erythroblast and C orthochromatic erythroblast. In cell A appears a Feulgen-negative nucleolus, surrounded by a thick layer of nucleolus-associated chromatin. In later development stages (B) only a compact chromocentrum can be seen. In the very last stages, the pycnotic nucleus (G) is entirely Feulgen-positive.

2. The ultraviolet-absorption curves from points in the cytoplasm of the promyelocyte (fig. 30) exhibit a maximum at 2600 Å, although a considerably lower one in comparison with data from preceding cells. The mean value of the quotients between the light absorptions at 2570 and 2750 Å in the promyelocyte-cytoplasms is at 1.20 (table 8).

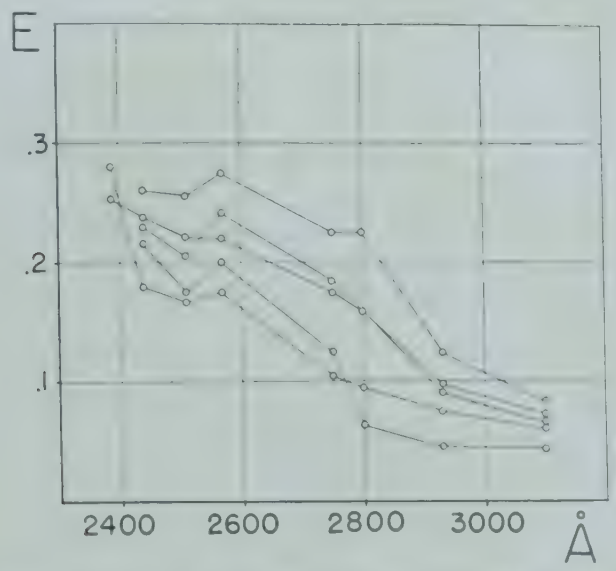


Fig. 30. Absorption spectra of points in the cytoplasm of promyelocytes.

Table 8.

The quotient $\frac{E_{257}}{E_{275}}$ *for points in the cytoplasm of promyelocytes.*

Cell	E_{257}	E_{275}	Q
1.....	0.350	0.285	1.22
2.....	0.265	0.220	1.20
3.....	0.380	0.310	1.23
4.....	0.190	0.160	1.18
5.....	0.255	0.210	1.20
6.....	0.235	0.200	1.17
7.....	0.420	0.350	1.20
8.....	0.570	0.460	1.24
9.....	0.300	0.250	1.19
10.....	0.130	0.110	1.18
11.....	0.275	0.230	1.20
12.....	0.470	0.385	1.22

$M = 1.20 \pm 0.01$

3. Cytoplasm and nucleoli are completely Feulgen-negative. The nucleolus-associated chromatin forms a fairly thick superficial layer, delimiting a mean area of 13 μ^2 (table 6).

From the light absorption data the cytoplasm can be estimated to contain about 3 % of polynucleotides with a proportion of c. 1 : 5 between polynucleotides and protein (fig. 29). The nucleotides in cytoplasm and nucleoli are of the ribose type.

c) *The myelocyte* (figs. 26 c, 27 c, 29, 31, tables 6, 9).

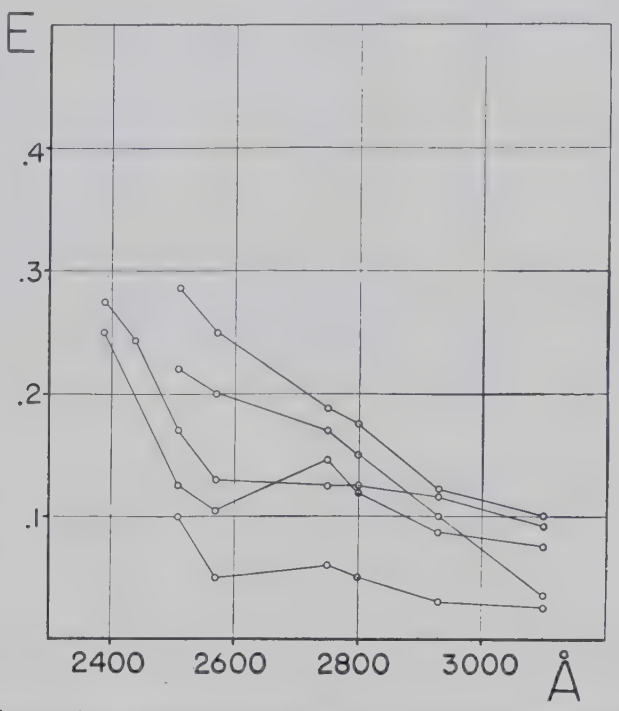


Fig. 31. Absorption spectra of points in the cytoplasm of myelocytes.

1. A round or oval cell with a mean diameter of about 13 μ (table 6). The cytoplasm is abundant, clearly granulated, the granula giving rise to unspecific losses of light. The nucleus is fairly small, oval or kidney-shaped. The ultraviolet-absorbing chromatin is collected in coarse granules. As a rule one or a couple of large nuclear bodies can be discerned in the nucleus.

2. The quantitative light absorption data are difficult to interpret owing to the unspecific loss of light caused by the light-dispersing granula. None of the curves have any maximum at 2600 Å (fig. 31). Some give an indication of a maximum at 2800 Å, probably due to the cytoplasmic protein. The quotient 2570 : 2750 Å is on an average 1 : 1, which must be considered the max. value, as in the majority of cases the absorption is affected unspecifically by the light dispersion of the granula (table 9).

3. The Feulgen-negative nucleolar mass in this cell has been reduced very considerably. The mean area is only 5 μ^2 (table 6). This is surrounded by a thick irregular superficial layer of nucleo-

lus-associated chromatin containing ribodesose polynucleotides (fig. 27 c).

Thus a great reduction has begun in the myelocyte, in comparison with the earlier cell types, as regards the concentration and amount of ribose polynucleotides, both in the cytoplasm and the

Table 9.

The quotient $\frac{E_{257}}{E_{275}}$ *for points in the cytoplasm of myelocytes.*

Cell	E_{257}	E_{275}	Q
1.....	0.110	0.125	0.88
2.....	0.155	0.140	1.11
3.....	0.065	0.067	0.97
4.....	0.182	0.170	1.07
5.....	0.200	0.175	1.14
6.....	0.195	0.175	1.12
7.....	0.250	0.210	1.20
8.....	0.180	0.165	1.09
9.....	0.185	0.195	0.95
10.....	0.135	0.135	1.00
11.....	0.460	0.400	1.12
12.....	0.670	0.540	1.20
13.....	0.463	0.445	1.05
14.....	0.170	0.190	0.90

$M = 1.06 \pm 0.03$

nucleolus. The light absorption data of the cytoplasm show that in general the concentration of ribose polynucleotides in the myelocyte does not exceed detectable amounts or 0.5 %.

d) *The granulocyte* (figs. 26 d, 27 c, 29, 32, table 6).

1. By way of the metamyelocyte the cell finally matures into the granulocyte. The cell becomes smaller, c. 10 μ in diameter, the cytoplasm is characterised by large quantities of light-refracting granula, and the nucleus passes from rod-shape to lobed. In some species of animals, such as rats and mice, this lobing takes place by way of a typical ring-shaped nucleus. In the mature white blood cell, the nucleus is sharply delimited, in it there are two or three strongly ultraviolet-absorbing bodies, usually distributed one to each lobe.

2. Owing to the deposits of strongly light-refracting granula the cytoplasm is characterized in ultraviolet by an unspecific light absorption increasing towards shorter wave lengths (fig. 32).

3. On the Feulgen-stained preparation, apart from the coarse

chromatin structures, there appear nuclear bodies as strongly stained ribodesose polynucleotide-containing chromocentra (fig. 27 c).

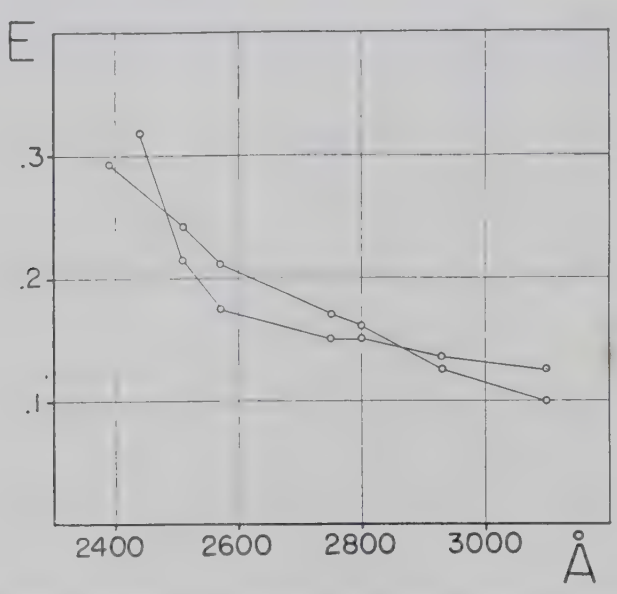


Fig. 32. Absorption spectra of points in the cytoplasm of mature granulocytes.

Thus the ribose polynucleotide-containing nucleoli have quite disappeared in the mature granulocyte and have left behind them chromocentra abundant in ribodesose polynucleotides. The low, entirely unspecific ultraviolet light absorption of the cytoplasm shows that the concentration of ribose polynucleotides has fallen to 0 ± 0.5 %.

2. Erythropoiesis.

a) *The proerythroblast* (figs. 33, 34 a, 35 a, 36, 37, tables 10 and 11).

Cellchemically this cell resembles the myeloblast described above and in the most unmature stage it cannot be distinguished from it. Somewhat later stages of more mature red stem cells can be distinguished from the myeloblast, as they have a coarser ultraviolet-absorbing chromatin and the even external contours typical of precursors of the red blood cells.

1. The cell is large, the meandiameter 12 μ (table 10). The cytoplasm absorbs light strongly at 2570 Å. In the nucleus there are one or two large, light-absorbing nuclear bodies (fig. 34 a).

2. The absorption spectra in ultraviolet of points in the cytoplasm and nucleoli have high maxima at 2600 Å (fig. 36). The mean quotient in the cytoplasm for $\frac{E_{257}}{E_{275}}$ is 1.26 (see table 11).

Table 10.

Cytometry of erythropoietic cells.[1]

Diameter μ		Nucleolus μ^2
Proerythroblast	12 ± 0.25 $\sigma = 1.6$ (50)	30 ± 0.33 $\sigma = 1.5$
Basophilic erythroblast	10 ± 0.30 $\sigma = 1.5$ (100)	—
Polychromatic erythroblast	8 ± 0.18 $\sigma = 1.1$ (150)	—
Orthochromatic erythroblast	6 ± 0.10 $\sigma = 1.0$ (100)	—

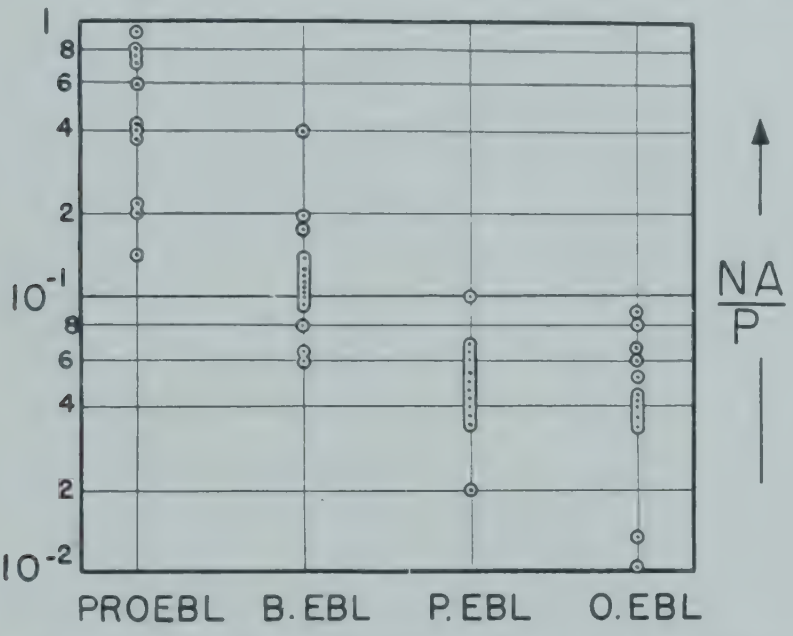


Fig. 33. The relation between the concentration of ribose polynucleotides and the concentration of protein in the cytoplasm during erythropoiesis. Calculations as in fig. 29. Proebl = proerythroblast, B. ebl. = basophilic erythroblast, P.ebl = polychromatic erythroblast and O.ebl = orthochromatic erythroblast.

Data obtained in the visible wave length range (fig. 37) show that substances of the porphyrin absorption type are not met with in detectable concentrations ($\pm$ 0.5 %).

3. The cell-picture after the Feulgen-reaction shows a large nucleolus, surrounded by ribodesose polynucleotide-containing

[1] The same precision as in the case of granulocytopoietic cells cannot be attained here, as the morphological transitions between the different stages are rather fluid. The values are mean values, obtained by measurements on ultraviolet-photograms of living cells. The figures in parentheses indicate the number of measured cells.

nucleolus-associated chromatin. In comparison with the nucleolus-associated chromatin of the myeloblasts, that of the proerythroblast appears to contain greater aggregations of chromatin (fig. 35 a).

Table 11.

The quotient $\frac{E_{257}}{E_{275}}$ *for points in the cytoplasm of proerythroblasts.*

Cell	E_{257}	E_{275}	Q
1.....	0.580	0.450	1.28
2.....	0.340	0.270	1.25
3.....	0.530	0.425	1.25
4.....	0.500	0.400	1.25
5.....	0.520	0.410	1.27
6.....	0.590	0.490	1.20
7.....	0.390	0.310	1.29
8.....	0.580	0.450	1.29
9.....	0.500	0.435	1.15
10.....	0.370	0.310	1.20
11.....	0.420	0.320	1.31
12.....	0.440	0.340	1.29
13.....	0.320	0.220	1.45
14.....	0.340	0.300	1.13

$M = 1.26 \pm 0.02$

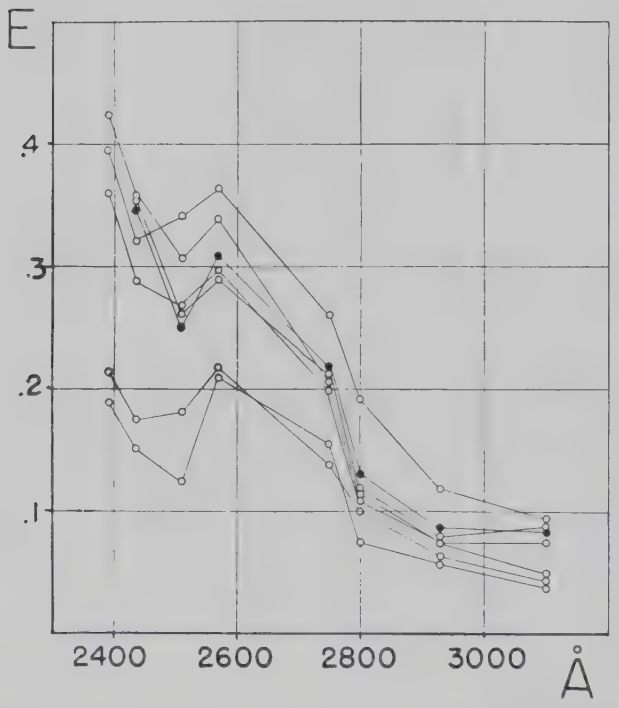
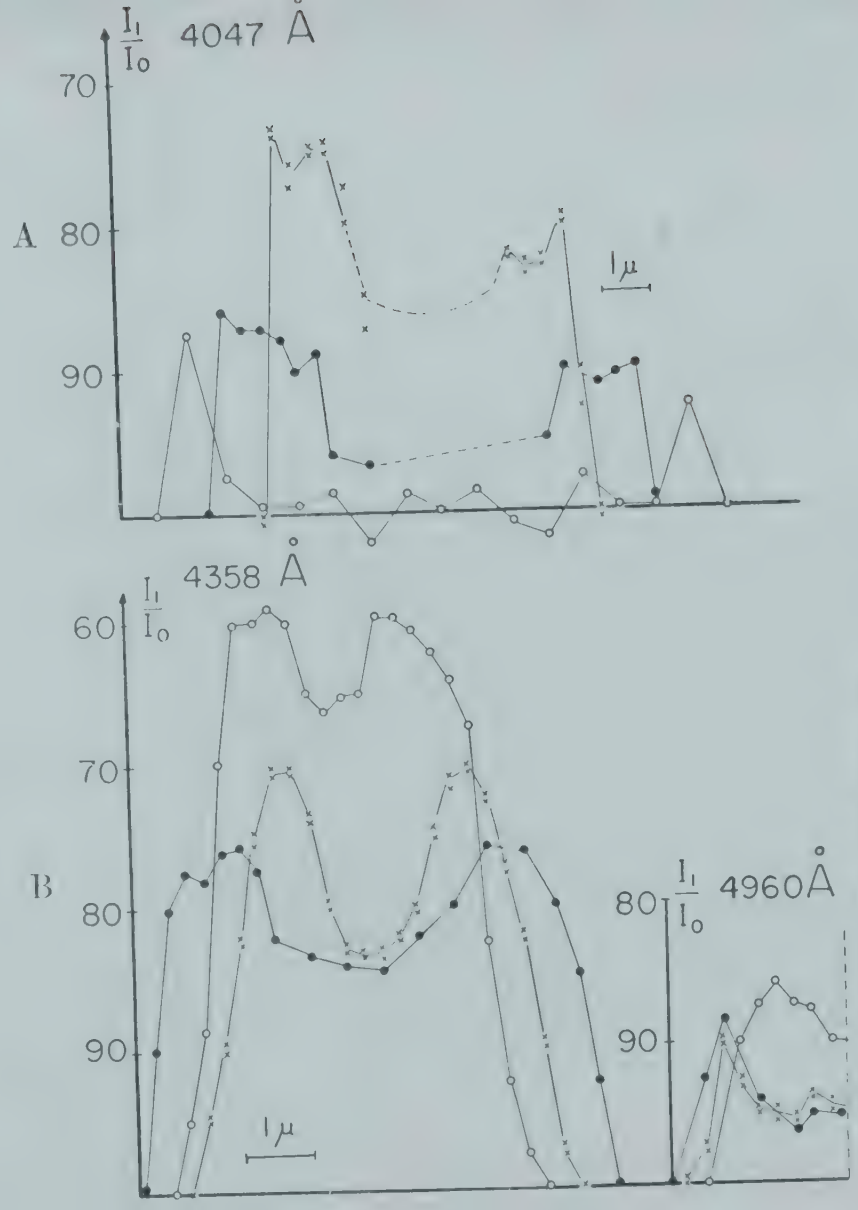


Fig. 36. Absorption spectra of points in the cytoplasm of proerythroblasts. The curve indicated by —•—•— is a spectrum from the identical cell in which absorption in visible light has been measured, (see fig. 37 a, curve —○—○—; also cell 10.5 μ in table 17).



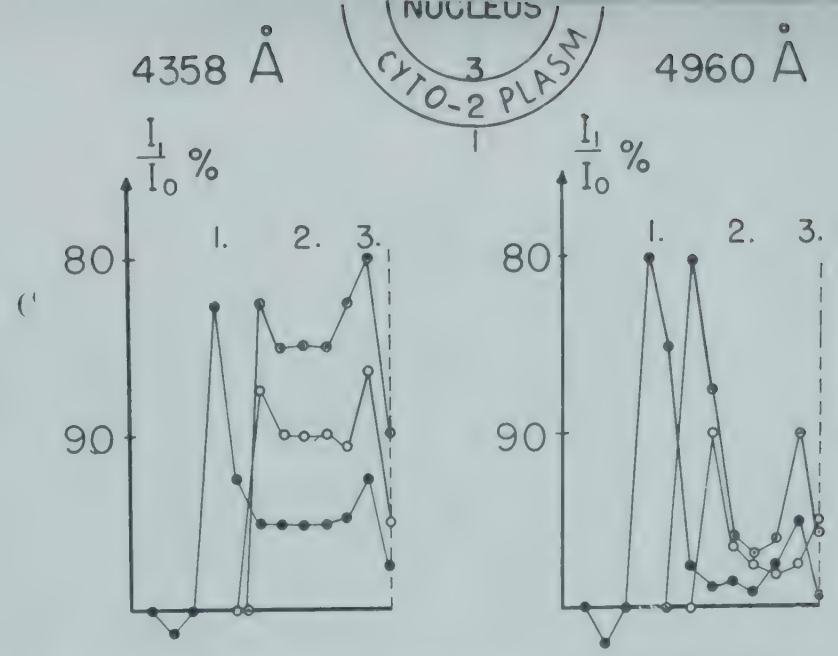


Fig. 37. Examples of absorption measurements according to the principle shown in fig. 23 of living erythropoietic cells in physiological saline solution.

A represents measurements at 4047 Å of early erythroblasts; proerythroblast 10.5 μ diam. —◡—◡—, and polychromatic erythroblasts, 8.5 μ diam. —•—• and 7 μ diam. —·—·—. The curve obtained from the proerythroblast shows only losses of light in the periphery of the cell due to refraction, cf fig. 5. Except for this and losses of light due to a few refracting chromatin granules, the cell is optically empty. In the polychromatic erythroblasts absorbing substances appear in the cytoplasm.

B represents measurements of erythroblasts with still more hemoglobin (orthochromatic). Because of the high cytoplasmic absorption in the Soret-band, the measurements are made at 4358 Å. The smaller figure shows measurements at 4960 Å from which the unspecific losses of light can be evaluated.

C represents measurements with higher resolving power of erythroblasts containing varying amounts of hemoglobin. The figures 1, 2, 3 indicate the localization of the measured points. The data show an equal distribution of hemoglobin in the measurable part of the cytoplasm in a given cell. For the values calculated from these measurements, see text and table 17.

The proerythroblast is thus characterized, as is the myeloblast, by comparatively high concentrations (c. 5 %) of ribose polynucleotides in the cytoplasm and nucleolus. From the quotients $\frac{E_{257}}{E_{275}}$ the ratio polynucleotides : protein can be calculated as 1 : 3 (fig. 33).

b) *The "basophilic" erythroblast* (figs. 33, 34 b, 35 b, 37, 38, tables 10 and 12).

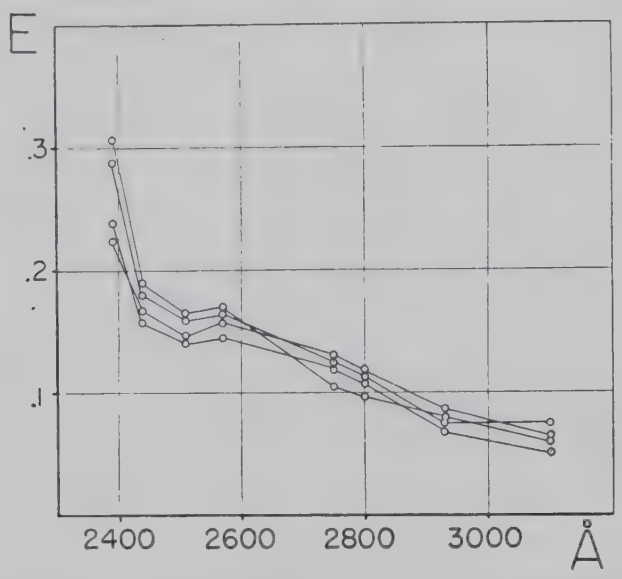


Fig. 38. Absorption spectra of points in the cytoplasm of basophilic erythroblasts.

1. The mean diameter of the cell is 10 μ. The cytoplasm absorbs ultraviolet moderately and does not appear fully so homogeneous as the preceding cells. In the round nucleus there are coarse, strongly absorbing grains of chromatin c. 1 μ in diameter, which are accumulated especially near the nuclear membrane. Further, there is a large irregular accumulation of chromatin in the centre of the nucleus.

2. The ultraviolet absorption spectra from points in the cytoplasm give an indication of a maximum at 2600 Å (fig. 38). The mean value of the quotient $\frac{E_{257}}{E_{275}}$ is 1.14 (table 12). The light absorption of the cell at 405 and 435 mμ is so low that the concentration of porphyrin derivatives cannot exceed 0.5 %.

3. After Feulgen's nucleal reaction the nuclear structures develop a colour strongly (fig. 35 b). In comparison with the ultraviolet absorption data the accumulation of chromatin must consist mainly of ribodesose polynucleotide-containing chromatin,

surrounding a small Feulgen-negative nucleolus situated in the centre. In comparison with the nucleolus of the preceding cell type this is inconsiderable however.

Table 12.

The quotient $\frac{E_{257}}{E_{275}}$ *for points in the cytoplasm of basophilic erythroblasts.*

Cell	E_{257}	E_{275}	Q
1.....	0.245	0.210	1.17
2.....	0.190	0.170	1.12
3.....	0.520	0.410	1.27
4.....	0.390	0.360	1.08
5.....	0.450	0.390	1.15
6.....	0.310	0.300	1.03
7.....	0.350	0.310	1.13
8.....	0.210	0.190	1.11
9.....	0.670	0.540	1.22
10.....	0.200	0.170	1.18
11.....	0.500	0.490	1.02
12.....	0.320	0.280	1.14
13.....	0.165	0.190	1.15
14.....	0.255	0.220	1.16

$M = 1.14 \pm 0.02$

The change from the preceding cell type thus consists mainly in a considerable decrease in the ribose polynucleotide-containing nucleolar mass and a decrease in the concentration of cytoplasmic ribose polynucleotides. The relation polynucleotides : protein may be calculated to be c. 1:7 (fig. 33). No observable amounts ($> 0.5\,\%$) of porphyrin derivative (hemoglobin) can be seen in the cell.

c) *The "polychromatic" erythroblast* (figs. 33, 34 c, 37, 39, tables 10 and 13).

1. The mean diameter of this type of cell is 8 μ (table 10). The light absorption of the cytoplasm at 2570 Å is strikingly small. Small vacuoles in the cytoplasm can be observed. The nucleus contains strongly condensed chromatin. A considerable chromocentre-like formation is met with in the centre of the nucleolus.

2. In the majority of cases the ultraviolet absorption of points in the cytoplasm has an entirely unspecific course (fig. 39). Some analyses indicate a protein band at 2800 Å. In view of the limits of the error of the determination, polynucleotide concentrations exceeding 0.5 % can be excluded. The mean quotient $\frac{E_{257}}{E_{275}}$ is at 1.0 (table 13).

The results of the measurements in the visible wave length range (fig. 37) show a clear porphyrin absorption. This is of an order of magnitude corresponding to 2 ± 0.5 % of hemoglobin. The total amount per cell can be calculated (see Chap. 3) to be about 3×10^{-6} μg.

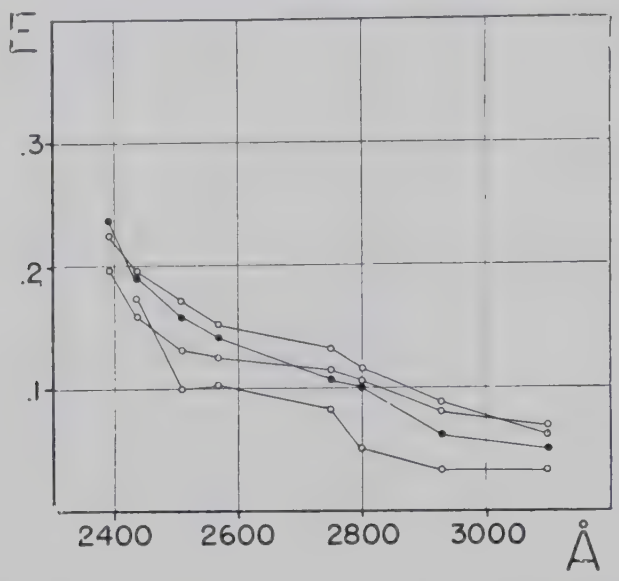


Fig. 39. Absorption spectra of points in the cytoplasm of polychromatic erythroblasts. The curve indicated by —•—•— is an ultraviolet absorption spectrum from the same cell which has been measured in visible light (see fig. 37 a, curve —•—•—).

Table 13.

The quotient $\frac{E_{257}}{E_{275}}$ *for points in the cytoplasm of polychromatic erythroblasts.*

Cell	E_{257}	E_{275}	Q
1.....	0.350	0.295	0.85
2.....	0.480	0.470	1.02
3.....	0.240	0.210	1.14
4.....	0.360	0.390	0.92
5.....	0.250	0.250	1.00
6.....	0.210	0.205	1.02
7.....	0.300	0.310	0.97
8.....	0.225	0.210	1.07
9.....	0.370	0.350	1.05
10.....	0.240	0.250	0.96
11.....	0.200	0.195	1.02
12.....	0.260	0.260	1.00

$M = 1.00 \pm 0.02$

3. After Feulgen's nucleal reaction the condensed chromatin of the nucleus strongly developed colour. No Feulgen-negative nucleolus can be discovered.

The decrease in the ribose polynucleotide concentration of the cytoplasm in this type of cell has continued down to $< 0.5\ \%$. The protein concentration may be estimated as between 15 and 20 %. The ribodesose polynucleotide-containing chromatin of the nucleus has been condensed, and the ribose polynucleotide-

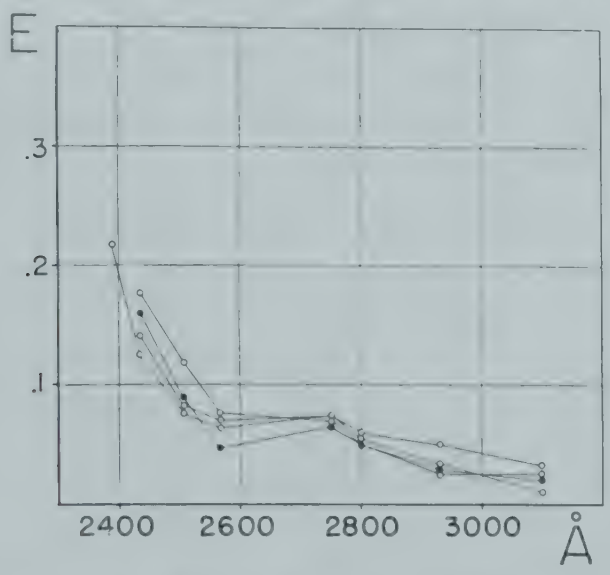


Fig. 40. Absorption spectra of points in the cytoplasm of orthochromatic erythroblasts. Curve —•—•— is a spectrum from the cell measured in the visible spectral range (see fig. 37 b, curve —○—○—).

containing nucleolar mass has disappeared entirely. At the same time traces of hemoglobin derivative (c. 2 %) is found in the cytoplasm of the cell.

d) The *"orthochromatic" erythroblast* (figs. 33, 34 d, 37, 40, tables 10, 14).

1. The mean diameter of the cell has decreased further down to 6 μ. The cytoplasm is slightly light-absorbing at 2570 Å and homogeneous. The nucleus is small with greatly condensed, strongly light-absorbing chromatin (pyknotic).

2. The ultraviolet absorption curve of points in the cytoplasm shows a pure protein absorption band (fig. 40). On account of the small dimensions of the cell it is difficult to analyse, but the quotients $\frac{E_{257}}{E_{275}}$ which are below 1.0, together with the absorption data in visible light (control of unspecific losses of light) indicate a total absence of nucleotides (table 14).

In the mercury bands 405 and 435 mμ there is a strong hemoglobin absorption (fig. 37) corresponding to a concentration of

between 20 and 25 %. Total amount can be calculated up to 28×10^{-6} µg.

3. After Feulgen's nucleal reaction only a pyknotic, compact Feulgen-positive mass of chromatin is visible (fig. 35 c).

Table 14.

The quotient $\frac{E_{257}}{E_{275}}$ *for points in the cytoplasm of orthochromatic erythroblasts.*

Cell	E_{257}	E_{275}	Q
1.....	0.110	0.110	1.00
2.....	0.160	0.200	0.80
3.....	0.100	0.105	0.98
4.....	0.120	0.120	1.00
5.....	0.300	0.260	1.11
6.....	0.200	0.195	1.02
7.....	0.100	0.130	0.77
8.....	0.255	0.240	1.06
9.....	0.300	0.300	1.00
10.....	0.350	0.310	1.12
11.....	0.255	0.250	1.02
12.....	0.170	0.170	1.00

$M = 0.99 \pm 0.03$

Finally, owing to a total loss of nucleus, the cell is transformed into a mature erythrocyte. The hemoglobin concentration, determined both macro- and micro-spectrographically in this cell, shows a final value of about 33 % (table 17). Total amount 28×10^{-6} µg. The increase in concentration is fully explained by the volume decrease (for further details see below, Chap. 1, Part II) from a spherical cell to the typical disc-shape of the red blood corpuscle.

3. Macrochemical Analysis.

In the blood-forming organ the different stages in cell development are intermingled. Therefore they are not open to ordinary macrochemical methods of analyses. In some special cases, however, macrochemical experiments can be carried out. If an experimental animal is rendered anemic by blood-tapping, for some time the blood-forming organ sends out more and more immature blood cells into the blood stream. Here those belonging to erythropoiesis can be obtained in quantities which permit of analyses by macrochemical methods. The latter will be compared with the above microspectrographical analyses.

a) *Analyses of hen's blood.*

For such experiments hen's blood is particularly suitable, as in this kind of animal immature cell forms are easily obtained (see WRIGHT and ALSTYNE, 1931).

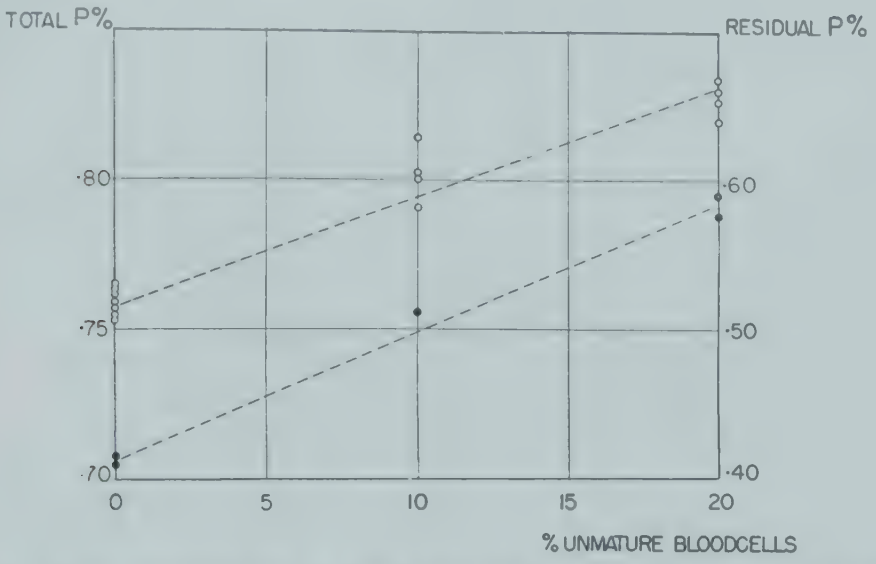


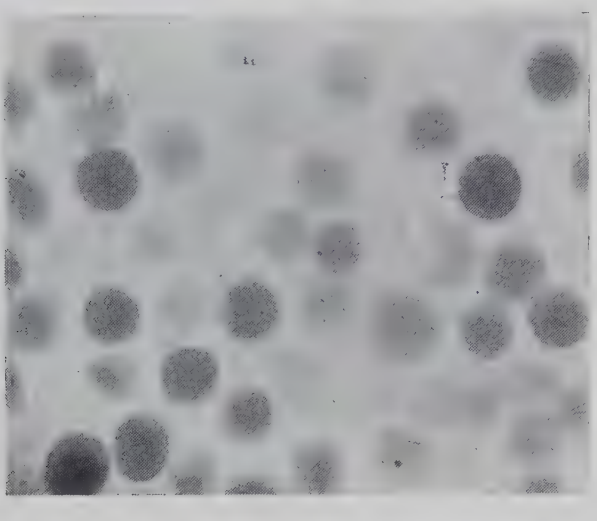
Fig. 41. Phosphorus analyses of blood samples (hen) containing different percentages of immature red cells. The phosphorus concentration (specifically the "residual phosphorus") is proportional to the percent of immature red cells in the samples. For details, see text.

By means of heart puncture 20—30 ml of blood were tapped off from 1—2 year-old white Leghorn hens every other day. The hemoglobin content and the number of red blood cells were determined in the usual way. The cytological picture was investigated, partly, in ordinary Giemsa-stained smears, and partly, in ultraviolet at 2570 Å. C. 10 ml of the blood tapped off was defibrinized with glass beads, the blood corpuscles were allowed to sink, after which serum and the white corpuscles were suctioned off. The remainder was washed with physiological saline solution. This was repeated 5—7 times. After the last washing the red blood cells were dried with alcohol and ether. The total phosphorus and the residual phosphorus (polynucleotide-P) after lipoid- and acid-extraction were determined by the micromethod indicated by TEORELL and NORBERG (1932). The values in fig. 41 were obtained.

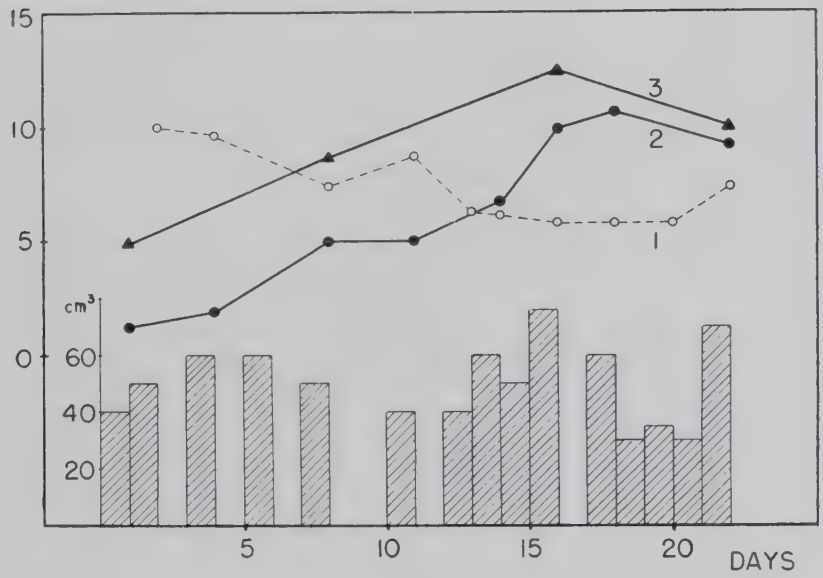
b) *Analyses of rabbit's blood.*

Rabbit's blood was used as the nucleus-free erythrocyte-material. Full-grown rabbits were tapped every other day (through the veins of the ears) of 40—60 ml. The cytological picture in the blood was examined in ultraviolet, and the light absorptions E_{257}

and E_{275} were determined. In no case were nucleated red blood cells observed. Instead the ultraviolet absorption of the blood cells



A



B

Fig. 42. A shows ultraviolet microphotographs taken at 2570 Å of polychromatic red cells from rabbit.
B shows analyses of red cells from rabbit. The vertical columns represent the amounts of blood withdrawn from the experimental animal. Curve 1 is the hemoglobin concentration in g/100 ml of the rabbit's blood. Curve 2 shows polychromatic red cells in %. Curve 3 is residual phosphorus of the blood samples in percent $\times 10^2$. The diagram shows, that the residual phosphorus (polynucleotide-P) is proportional to the percent of polychromatic red cells.

increased at 2570 Å, fig. 42. From the absorption data these "polychromatic" blood cells could be estimated to contain between 0.5 and 1 % of nucleotides. The blood cells were washed and dried

as above, after which the residual phosphorus was determined. For the values, see fig. 42.

On some specimens with varying contents of ultraviolet (2570 Å) absorbing erythrocytes, the ribose polynucleotide-P and the mononucleotide-P were determined after extraction according to HAMMARSTEN (1947), the values in table 15 being obtained.

Table 15.

Ribose-polynucleotide phosphorus (in percent of dry weight) in immature erythrocytes, estimated after extraction according to HAMMARSTEN 1947.

% immature	Polynucleotide-P		Low molecular weight nucleotides-P
	extracted	non-extractable	
1	0.002	0.042	0.003
5	0.017	0.063	0.001
10	0.024	0.083	0.004
10	0.027	0.070	0.004

The results show that, parallel with the increase in the number of immature blood cells (hen's blood) and at 2570 Å light-absorbing ("polychromatic") unnucleated rabbit erythrocytes, an increase is obtained in polynucleotide-phosphorus, determined macrochemically. This is in good agreement with the above light absorption analyses of the individual cell elements of erythropoiesis.

CHAPTER 5.

The Endocellular Growth Mechanism During the Blood Cell Formation.

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The primary problem formulated at the end of Chapter 2 is: Does the new-formation of cytoplasmic protein during the blood cell production proceed in the presence of polynucleotides, and with a cytochemical organization resembling that of other growing cells, *e. g.* embryonal cells (Caspersson and Thorell, 1941), and further, are the effects of these metabolisms during the normal continuous blood cell renewal in the adult of such an order of magnitude that they are suited to quantitative cytochemical methods of analysis?

With the help of the cytochemical polynucleotide analyses in Chapter 4, in this chapter it will be investigated whether the new-formation of proteins during the blood cell production can be correlated with an endocellular ribose polynucleotide metabolism of the cell. In this connection our intention is to investigate whether the principal increase in cell substance during the blood cell formation is linked up with a certain phase of development, and whether, during this (eventual) phase of development, the cell has an organization characterized by comparatively high concentrations of ribose polynucleotides in the cytoplasm and in the nucleolar apparatus.

A. Cell Growth During Granulocytopoiesis.

1. Comparison of Analytical Data.

The changes in the chemical composition of the cell during granulocytopoiesis are seen most clearly if the analytical data are put together along a suitably divided axis, showing the relative chronological order of the different types of cells. An appropriate basis of division is the percentage of the above-defined cell types of the whole number of cells in bone marrow. This changes measurably during the development and has the advantage that it has no direct connection with the properties of the cell. In this way curve 1 (the nucleolar mass) and curve 2 (the cytoplasmic polynucleotide concentration) are obtained in fig. 43.

These changes in the endocellular concentration and distribution of ribose polynucleotides will be correlated with the changes in the cellular protein during granulocytopoiesis.

During granulocytopoiesis the formation of new cellular substance gives rise partly to a change in the number of cells, and

Table 16.

The average cellular volumes and the frequency of each cell type in the normal bone marrow (Human and rat).

	Diameter μ	Cellular volume μ^3	%[1]	"Volume ratio" (see text p. 74)
Granulocytopoiesis:				
Myeloblast	13.3	1,200	1(0—2.4)[2]	1 : 8
Promyelocyte	16.3	2,300	4(1—9.5)	1 : 1
Myelocyte	12.9	1,100	10(4.5—18)	1 : 1
Mature granulocyte	10.2	550	20(10—30)	
Erythropoiesis:				
Proerythroblast	12	900	1[3]	1 : 7
Basophilic erythroblast	10	500	12	1 : 1
Polychromatic erythroblast	8	250	15	1 : 1
Orthochromatic erythroblast	6	100	25	

[1] Mean values of estimations on ultraviolet photographs (Σ 5000 cells). The granulocytopoietic cells were identified by their ultraviolet cytological picture and the erythropoietic cells by their diameter. The values obtained agree on the whole with those from earlier investigations on the cellular composition of the bone marrow. (For a review see Osgood and Seaman 1944, where the sources of error are also discussed).

[2] The figures in parentheses indicate the variation ranges of the results in earlier investigations.

[3] These values can hardly be compared with those from the literature due to the badly defined cell types.

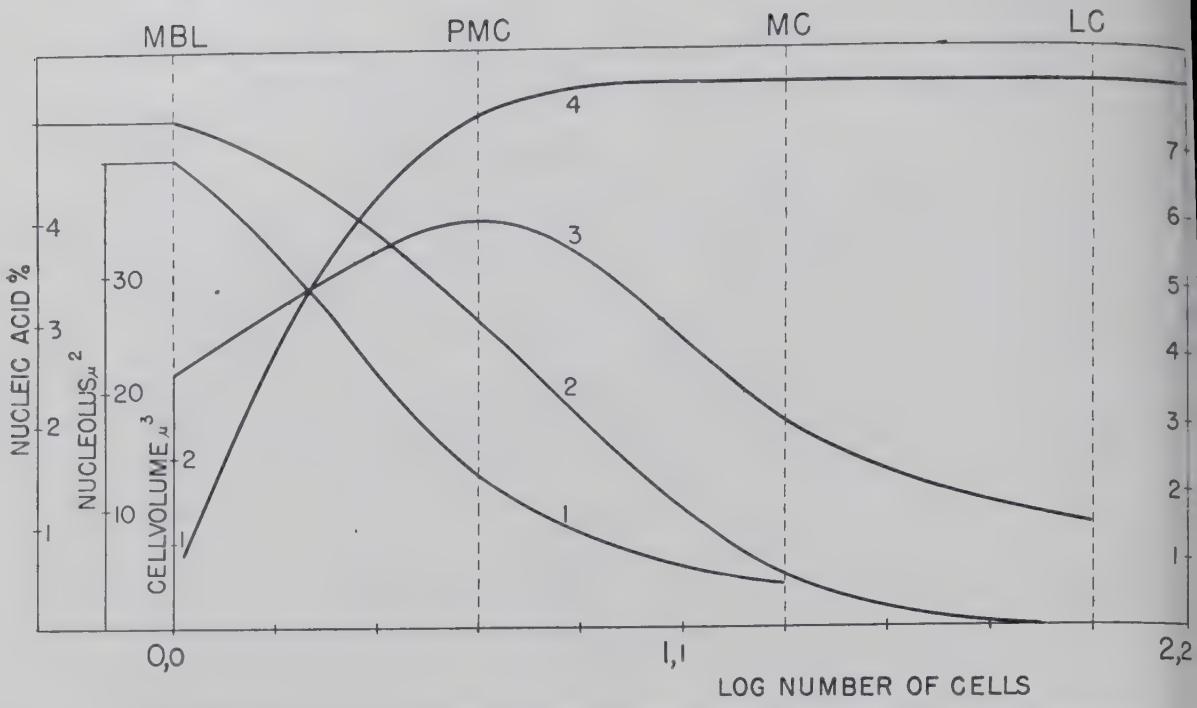

Fig. 43. Survey of some essential cytochemical and cytological changes connected with the endocellular growth processes during granulocytopoiesis. MBL = myeloblast, PMC = promyelocyte, MC = myelocyte and LC = mature granulocyte. The curves are: 1 optical cross section area of the nucleoli in the cell, 2 cytoplasmic ribose polynucleotides in %, 3 volume of the cell, 4 total volume of cells in each development phase.

From the diagram it appears that before and during the principal increase in cellular mass (c. 8 ×) the granulopoietic cells are characterized by a large nucleolar mass and high concentrations of ribose polynucleotides in the cytoplasm. During the course of maturation the nucleolar mass and the cytoplasmic ribose polynucleotides decrease parallel with a cessation of the growth activity of the cell. For details, see text.

partly to a change in cell volume. This cell development can be divided into stages according to certain principles, appropriately into the four type phases as above: myeloblast, promyelocyte, myelocyte and granulocyte. The author's own data, together with statements of earlier authors as to the *percentual occurrence* of the respective cells (see Osgood and Seaman, 1944), are shown in table 16.

It was shown above (see table 1, Chap. 3) that, with the preparation technique employed, the bone marrow cells may be regarded as approximately spherical. Thus from the diameters in table 16 the *volumes* of the different cell forms can be calculated (curve 3, fig. 43).

Each phase of development is represented by a total cell volume which is obtained if the number of cells in the phase in question is multiplied by the cell volume characteristic of the phase. *Thus*,

as an expression of the quantitative changes in the cell mass during granulocytopoiesis, the relation between the total volume of the different stages of development can be employed (curve 4, fig. 43) (see also THORELL 1944, p. 360). If this relation is calculated, it is found that the total volume of myeloblasts to that of promyelocytes is: c. 1 : 8. During later phases of development, promyelocytes : myelocytes : granulocytes, the total volume is broadly speaking *constant*.

If it is to be possible to employ the relation between the cell volumes as a measure of the changes in the cell protein, it is necessary that the protein concentration within the cell should be relatively constant. As the water content in tissues in general is fairly constant (80 $\pm$ 10%, see HAMMARSTEN, 1910) considerable changes, such as the doubling of the volume owing to water-uptake, appears improbable. The results of the ultraviolet absorption analyses in the preceding chapter also exclude variations in the protein concentration exceeding values between 15—20%. As in this case it is a matter of changes amounting to 8 times, the possibility of such a small variation is negligible in this connection.[1]

2. Changes in the Cellular Ribose Polynucleotide Metabolism During Granulocytopoiesis.

The earliest stem cell in the scheme, the myeloblast, with a frequency of c. 1 %, is characterized by a high concentration of cytoplasmic polynucleotides (> 5 %) and a large, ribose polynucleotide-containing nucleolar mass. Already in the following stage of development, the promyelocyte (c. 4 % frequency), the ribose polynucleotide-containing nucleolar mass has decreased considerably from an optical cross-section area of 39 μ^2 to 13 μ^2. The concentration of cytoplasmic polynucleotides has fallen from 5 to 3 %. As, during the transition from myeloblast to promyelocyte, the total cell volume has increased c. 8 times, this implies a total increase in cytoplasmic polynucleotides of between 4—5 times. This new formation of polynucleotides can hardly be calculated more accurately, on account *inter alia*, of the inhomogeneity of the cytoplasm. In the *individual* cell, however, a considerable reduction in the concentration and amount of ribose polynucleotides has taken place with the maturation into the promyelocyte.

[1] Later experiments (THORELL 1947) on ultramicro-refractometrical determining of the endocellular protein concentration during hematopoiesis with the use of the phase contrast principle did not show any changes exceeding the limit of the method (25 % of estimated value).

During the subsequent maturation which is characteristically reflected cytologically in the changing shape of the nucleus and in the appearance of granula, both the nucleolar mass and the cytoplasmic ribose polynucleotides are rapidly reduced. Apart from the continuous decrease in the concentration of ribose polynucleotides in the individual cell, already commencing with the maturation of the myeloblast, the absolute amount of ribose polynucleotides also decrease rapidly.

From the curves in fig. 43 it appears that it is *before* and *during* the principal increase in cellular substance (8 times) that the cell contains a large ribose polynucleotide-containing nucleolar mass and high concentrations (> 5 %) of ribose polynucleotides in the cytoplasm.

During the later phases of cell development, the total cell volume undergoes no, or only slight, changes; only the number of cells becomes larger. Every cell division thus takes place at the expense of the cellular volume. During this phase of development the cytoplasmic ribose polynucleotides rapidly disappear. Already at the stage of development defined as myelocyte, the cytoplasmic polynucleotide concentration is 0 ± 0.5 %. No ribose polynucleotide containing nucleolar mass can be detected in later development stages.

From these facts — that in the earlier stages of development the organization of the cell is characterized by high concentrations of ribose polynucleotides in the cytoplasm and nucleolar apparatus, and that these properties can be correlated with the vastly predominating increase in cell substance during granulocytopoiesis — it follows that the cellular protein formation of the granulocytic elements takes place in a similar way as has been shown earlier for other kinds of cells, *e. g.* embryonal cells (see Chap. 2).

During maturation the activity of the cell in protein formation decreases parallel with a continuous decrease in the concentration of ribose polynucleotides in the cytoplasm and in the nucleolar mass. In the later stages, myelocyte-granulocyte, the endocellular mechanism for the new-formation of the fundamental cell protein substances is entirely inactive. The relation may be expressed thus: the more the myeloid cell matures towards its final function, the more it loses its capacity for growth.

As shown above, this decrease in the intensity of the ribose polynucleotide metabolism can be analysed in detail owing to the accessibility of bone marrow cells in the living state. From this,

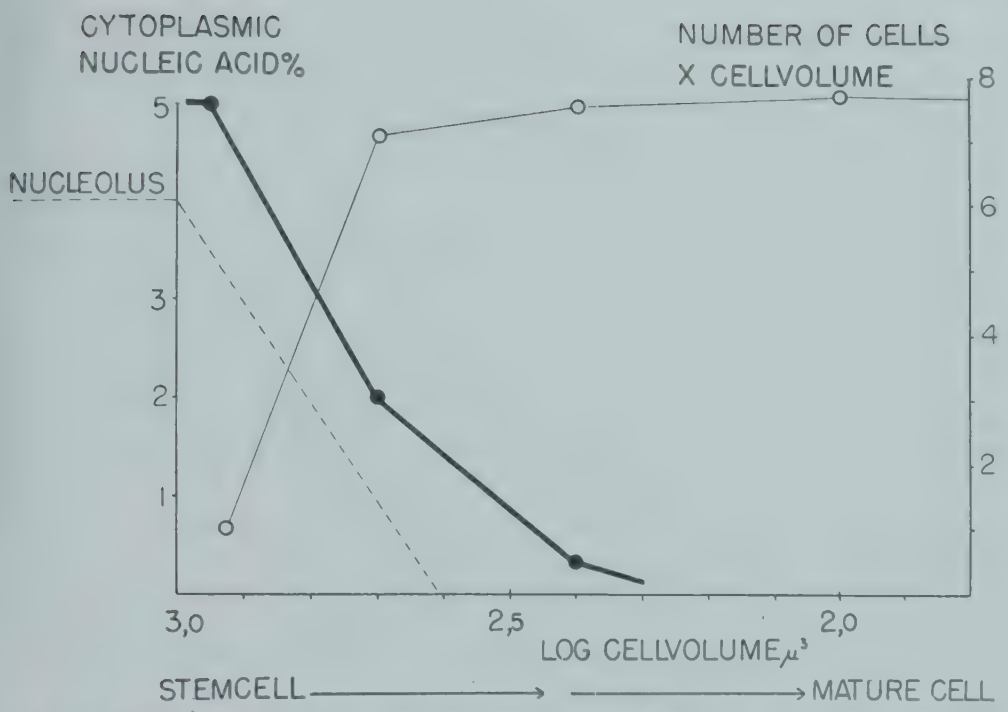

Fig. 44. Survey of some essential cytochemical and cytological changes connected with the growth processes during erythropoiesis. Abscissa: logarithm of cellular volume. (As can be calculated from the values in table 16, the log cellular volume of proerythroblasts is c. 2.95, basophilic erythroblast 2.70, polychromatic erythroblast 2.49 and orthochromatic erythroblast 2.0.) Under certain conditions the **log cellular volume is proportional to time.**

The figure shows essentially the same phenomenon as does fig. 43, namely that it is only before and during the vastly predominating increase in mass (number of cell × cell volume), that the erythropoietic cells are cytochemically characterized by a large nucleolar mass and high concentrations of cytoplasmic ribose polynucleotides. **For details, see text.**

there follows an alternative possibility of determining the growth conditions of an individual cell by analysis of the cytoplasm and nucleolar apparatus. In contrast to, *e. g.* the frequency of mitosis, which has earlier been used as a measure of the cellular growth activity (see Chap. 1), this basis of appraisement has a direct connection with the metabolism which is correlated with the new-formation of cellular substances.

B. Cell Growth During Erythropoiesis.

Erythropoiesis can be studied in the same way as indicated above (fig. 44). In its cytological properties, the earliest cell in the development, which in hematological literature is called the proerythroblast, is very like the stem cell of the granulocytopoiesis. The cell is relatively large and has a large nucleus-cytoplasmic ratio, c. 5 % of ribose polynucleotides in the cytoplasm and one

or two large ribose polynucleotide-containing nucleoli, surrounded by a thin superficial layer of nucleolus-associated chromatin.

During erythropoiesis the volume of the individual cell elements undergoes a continuous decrease. If the respective total volumes of the different stages of development are calculated, a relative increase is met with only in the very first stages (fig. 44), as in the granulocytopoiesis. It appears from the microspectrographical analysis data that the concentration of ribose polynucleotides in the cytoplasm undergoes a decrease from 5 to 2 % in the very first cell generations. Immediately after the phase of development, which morphologically is called "polychromatic", the zero value of cytoplasmic ribosepolynucleotides is reached. In the same manner, the ribose polynucleotide-containing nucleolar mass disappears very early, leaving behind it a compact chromocentre.

Thus, during erythropoiesis also, the predominant increase in cell substance can be correlated with an endocellular organization characterized by high concentrations of ribose polynucleotides in the cytoplasm and nucleolar apparatus.

C. Comparison Between the Cell Development During Granulocyto- and Erythropoiesis.

The above survey of the changes in the distribution of ribose polynucleotides during granulocytopoiesis and erythropoiesis show a similar course on the whole, which can be interpreted as a continuous decline during maturation of the activity of the individual cell in the formation of proteins.

Cells of very similar type in cytochemical respects (see above) function as the stem cell for both erythropoiesis and granulocytopoiesis. The similarity is emphasized also in purely morphological descriptions, *e. g.* Maximow's hemocytoblast, Pappenheim's lymphoidocyte, Naegeli's macroblast, etc.

If the development in the two series after the stem cell is compared, however, a striking difference appears in the development of the cytoplasms. Except for the very earliest cell, the erythroid cell series is characterized by relatively small amounts of cytoplasm, while the latter is abundantly developed during the myeloid cell series (see tables 6, 10, fig. 45).

The above cytochemical analysis shows that the decline in the concentration of cytoplasmic polynucleotides and in the nucleolar apparatus takes place more rapidly in the red series than in the white. Earlier authors, *e. g.* Downey, also describe how the acido-

philic nucleoli, typical of the stem cells, disappear earlier in the red development series than in the white, where they can be observed right up to the myelocyte stage.

From the fact that the cells in the myeloid series show large cytoplasms, whereas those in the erythroid series are comparatively small, it follows that the production of cytoplasmic proteins during the growth of the former cells proceeds with greater intensity than in the latter. If we correlate with this fact the above described cytochemical analyses of the ribose polynucleotides in the cells of the two series, it will be found that the heavier production of cytoplasmic proteins in the myeloid cells runs parallel with an evidently well-developed nucleolar apparatus, whereas in the erythroid cells — in conformity with the less marked formation of new cytoplasmic protein —, such an apparatus is well developed only in the very earliest stages, and subsequently disappears.

D. Summary.

The results reached in Chap. 5 may be summarized as follows: By comparing the analysis of the distribution and concentration of the polynucleotides in the bone marrow cells with the quantitative cytology of the bone marrow, it can be shown that the new-formation of cellular proteins during hematopoiesis takes place at an early stage of development in the presence of high concentrations (> 5 %) of ribose polynucleotides in the cytoplasm and nucleolar apparatus, *i. e.* with an endocellular organization similar to those of growing cells investigated earlier, *e. g.* embryonal cells (Caspersson and Thorell, 1941).

During cell-maturation the ribose polynucleotide-containing nucleolar mass and the concentration of cytoplasmic polynucleotides decrease continuously to 0 ± 0.5 % parallel with a declining growth activity of the individual cell.

The possibilities of microspectrographical and cytological quantitative analysis of bone marrow cells in the living state are so favourable that the results firstly form a strong support for the conception of the mechanism for the new-formation of cytoplasmic proteins (see Chap. 2), and secondly the results afford possibilities of appraising the growth state of an individual cell by means of analysis of the endocellular ribose polynucleotide concentration and distribution.

CHAPTER 6.

The Relationship of the Cellular Growth Processes to the Gene Material.

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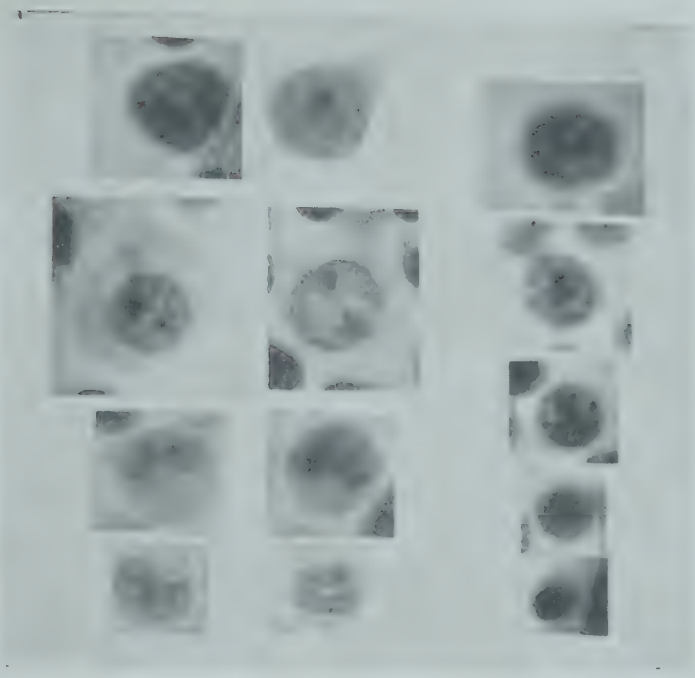
In this chapter it will be investigated if it is possible to trace the above described (Chap. 5) changes in the cellular ribose polynucleotide metabolism during cell-maturation back to a primary regulation mechanism within the gene material. This is especially of interest for the analysis of pathological disturbances in growth (Part III).

A. The Function of the Heterochromatin.

Works by Caspersson and Schultz on Drosophila material have shown that the metabolism of the ribose polynucleotides in these cells is controlled by definite chromosome regions, namely *the heterochromatin*. Their results show, *inter alia*, that the heterochromatic regions in *Drosophila melanogaster* have the capacity to form and affect the composition of the nucleoli and thereby influence the ribose polynucleotides in the cytoplasm of the egg (see Chap. 2, C).

The cytological and cytogenetic facts deriving from specially suitable material which support the heterochromatin conception can be summarized as follows (Heitz, 1935, Geitler, 1938, White, 1940): They are parts of the chromosome material, which in many cases are stainable in all phases of the cell cycle. In the few cases which have been investigated the sex chromosomes seem to have an abundance of such



A B

Fig. 45. Comparison between the granulocytopoietic (A) and the erythropoietic cells (B). Living cells photographed at 2570 Å. Magnification 1150 ×
From the comparison it appears that the development of the cytoplasms is more abundant in the granulocytopoietic series than in the erythropoietic.

A B C

Fig. 46. Telophase-interphase cells from a section of the root of guinea pig incisor. Feulgen reaction. Magnification c. 3000 ×.
A telophase, B late telophase and C interphase. Note the development of the nucleolus-associated chromatin. For details, see text.

A B C

Fig. 47. Ultraviolet microphotographs taken at 2570 Å of mitoses of living granulocytopoietic cells. The individual pictures are equally photographed, copied and printed. A myeloblast, B early myelocyte and C late myelocyte. The pictures show a dissimilarity in density of the chromosomes of cells in different maturation stages.

regions. Instead of being stainable in all phases, they may, under certain conditions, be relatively under-stained, at the same time as the euchromatic parts are strongly stainable. The heterochromatic regions are often indiscriminate in their pairing capacity. Nucleoli are often formed in association with them. They also appear to lack gene properties, which directly affect morphological characteristics.

In Drosophila, where it is possible to compare the chromatin regulating the new-formation of proteins with the cytologically and genetically defined heterochromatin, they agree. Thus in this chromosome material there are definite regions which, in functional respects, are specifically directed towards guiding the endocellular organization of the new-formation of proteins.

In the case of higher mammal material, however, the cytological and genetic definition of the heterochromatin is not yet possible. Therefore it is impossible to decide, whether the parts of the chromatin which are associated with the mechanism for protein new-formation and the heterochromatin are comparable.

B. The Relationship of the Nucleolus-Associated Chromatin to the Cellular Ribose Polynucleotide Metabolism.

The analysis data given in Chap. 4 present a possibility of investigating on mammalian and human material the relation between chromatin substance and the cellular ribose polynucleotide metabolism connected with the new-formation of proteins. During blood cell maturation a continuous decline takes place in the cellular growth activity, which decline, especially during granulocytopoiesis, takes place relatively slowly and can be followed by measuring certain cytochemical processes. These will be correlated below with the cytological phenomena in the nucleus during blood cell maturation.

By using living cells for the ultraviolet microphotographs and by treating the Feulgen stained preparations cautiously (fixation with formalin fumes), good pictures are obtained of the ribose- and ribodesose polynucleotide-containing parts of the nucleus. In this way the development of the nucleolus and especially of the nucleolus-associated chromatin can be followed in detail during the phases of cell maturation.[1]

The earliest cell in the development series, the myeloblast, has

[1] For a review concerning earlier morphological investigations on the development of the nucleolus during hematopoiesis, see Thorell 1944, p. 340.

a high concentration of cytoplasmic ribose polynucleotides and has two to four nucleoli in the nucleus, which the analysis shows to contain considerable amounts of ribose polynucleotides. After Feulgen's nucleal reaction the nucleolus-associated chromatin surrounding the nucleoli stands out (fig. 27). By measuring the optical cross-section area of the volume surrounded by the nucleolus-associated chromatin, a measure is obtained of the nucleolar mass. (See above p. 53.) In the myeloblast the mean figure for the latter is 39 μ^2 (see table 6).

In the next phase of development, the promyelocyte, where the concentration of cytoplasmic ribose polynucleotides has fallen to about half, the nucleolar mass has decreased to 13 μ^2.

The nucleolus-associated chromatin surrounding the nucleolus appears in the Feulgen-stained preparation as an irregularly thickened outer-layer.

With the maturation to the myelocyte the cytoplasmic ribose polynucleotides disappear almost entirely. The nucleus begins to become rod-shaped, the chromatin aggregates in the interphase, and the small, hardly observable ribose polynucleotide-containing nucleoli are surrounded by a thick layer of nucleolus-associated chromatin.

In the mature granulocyte no ribose polynucleotide-containing nucleolar mass is formed: from the point of view of growth the nucleolus-associated chromatin lies inert as an irregular, compact, Feulgen-positive chromocentre.

The central part in the mechanism for protein new-formation, the nucleolar substance, thus stands in direct connection with ribodesose polynucleotide-containing chromatin. The relation between the nucleolus types of the different phases of development is most simply explained by the supposition that the nucleolus-associated chromatin in the different stages is less and less disrupted by successively decreasing production of ribose polynucleotide-containing nucleolar substance during the interphase.

Thus both the heterochromatin in Drosophila and the nucleolus-associated chromatin in the bone marrow cells can be correlated with the cellular ribose polynucleotide metabolism. This similarity in respect of such a general cell function as the new-formation o cytoplasmic proteins during growth argues in favour of a conformity between these two conceptions.

C. The Relationship of the Nucleolus-Associated Chromatin to the Chromosome Material.

To determine directly the relationship of the nucleolar apparatus to the chromosome material presents great difficulties, chiefly because, in the phases of the cell cycle, when the gene material is accumulated into observable chromosomes, the nucleoli are disintegrated. The conditions are reversed during the interphase, when the nucleoli stand out but the chromosomes are not observable. A possibility would be to investigate the part of the telophase during which the chromosomes are disintegrated, and the interphase nucleus is formed. For this, however, a material with many mitotic divisions in a small area is required. Bone marrow is less suitable, owing to the relatively slight frequency (1 %) of the nucleolus-forming stem cell. Rodent teeth with so-called open and continuously growing roots are more appropriate. As the material originates from the same group of animals, it will be justifiable to assume that, in principle, conditions are the same during the formation of new dentine cells as in the blood cell formation, especially in view of the fact that it is a matter of a general cell-function: the new-formation of cytoplasmic proteins during the growth phase. In the rodent incisors, growth is restricted to one definite small area, in which the cells are in a state of active division. As THORELL and WILTON (1945) have shown, the root cells, like growing cells in general, are characterized by high concentrations of ribose polynucleotides in cytoplasm and nucleoli.

Fig. 46 shows cells from a section of the root of a tooth stained with Feulgen's nucleal reaction. In these cells it appears clearly that during the telophase, some of the chromosome material accumulates into ball-like formations in the central parts of the nucleus. During the following phase nucleoli appear inside these formations. This demonstrates the direct connection between parts of the chromosome material and the chromatin associated with the formation of nucleoli.

The results under B and C may be summarized as follows: 1. During blood-cell maturation the nucleolar apparatus is in a quantitatively definite relationship to the cellular ribose polynucleotide metabolism. 2. In the growing immature cell the formation of the nucleoli is directly connected with certain parts of the

chromosomes. The latter parts are defined during the interphase as nucleolus-associated chromatin. This indicates that, during the processes of the formation of new cellular substance, the growth mechanism is primarily controlled by these parts of the gene material.

D. The Nucleolus-Associated Chromatin and the Mitoses of Bone-Marrow Cells.

During the mitosis, when the nucleoli and nuclear membrane have disappeared, the factors which chiefly determine the cell function, the gene material, are accumulated in the chromosomes rich in ribodesose polynucleotides.

Ellerman (1923, 1924) and Petri (1926) describe a characteristic difference between the pictures of the mitoses of the myeloid and erythroid precursors: The angle of the anaphase spindle is c. 3 times as large in the former. La Cour (1944) also shows typical changes in the cytological picture of the chromosomes during the cell development of the same kind of cell.[1] Fig. 47 shows ultraviolet microphotographs taken at 2570 Å of the metaphase chromosomes in the case of granulocytopoiesis in the rat. A pronounced dissimilarity in density is evident from the pictures. During maturation the length and breadth of the chromosomes decrease at the same time as the light absorption per unit of surface increases. However, an interpretation in quantitative data of these changes is not practicable by the present methods.

In conjunction with the above account of the changes in the nucleolar apparatus during blood-cell maturation and Caspersson's and Schultz's investigations on Drosophila salivary gland cells (see Chapter 2, C) this observation indicates that the change in function during the cell maturation in the chromatin parts, which, during the interphase, constitutes the nucleolus-associated chromatin during mitosis also affects the chromosomes' metabolism of ribodesose polynucleotides!

E. The Cause of the Functional Change in the Nucleolus-Associated Chromatin During Blood Cell Maturation.

The change in the chromatin-regions which regulate the endocellular growth processes during hematopoiesis can in principle take place in two different ways: either as a decrease in the amount of functionating chromatin, or as a decrease in the activity of quantitatively unchanged substance. In the former case the change will probably be irreversible, in contrast to the latter case This distinction is of interest, especially for the question of the

[1] Biesele has recently found (1947) in malignant growing mouse leukaemia a 2- or 3-fold increase of the chromosomal mean volume compared with the chromosomes of normal lymphocytes.

cytogenetic connection between pathologically and normally growing blood-cells.

In principle the first alternative is conceivable since, unlike the euchromatin, the homologen (in the cytochemical sense) of this chromatin in Drosophila appears to be built up of identical or similar gene elements (Caspersson, 1941). In the Drosophila egg an addition, in the form of an Y-chromosome, increases the concentration of ribose polynucleotides, both in the cytoplasm and in the nucleolus (Schultz et al., 1940). In Solanum pollen, too, the addition of extra heterochromatin increased the size of the nucleolus (Lesley, 1938). Examples of reverse conditions are also met with. In Ascaris, Dysticus, etc. the sex cells contain large amounts of chromatin, which are eliminated in the somatic cells.

In the most extremely differentiated blood cell, the red blood corpuscle, circulating in the blood stream, all the chromatin has been eliminated. In the most differentiated granulocytes also an elimination of chromatin appears to take place through the extrusion of "heterochromatin" from the nucleus to the cytoplasm (La Cour, 1944). According to the analyses above, however, the growth activity of the bone-marrow cells has ceased at far earlier stages (myelocyte and basophilic erythroblast respectively). During the decline in growth from the stem cell, the cytology of the nucleolus-associated chromatin shows no signs of decreasing in quantity. In the relatively mature myelocyte a considerably thicker superficial layer — in comparison with the myeloblast nucleolus — of Feulgen-positive nucleolus-associated chromatin can be observed. During the subsequent maturation the latter changes into a compact chromocentre (fig. 27).

From this it follows that the change in growth intensity during cell maturation might be reversible. Thorell and Wilton (1945) showed that the normal dentin cell differentiation proceeds, as far as the ribose polynucleotide metabolism is concerned, in a manner similar to the differentiation of the blood cells, *i. e.* a decreasing intensity in the new-formation of cytoplasmic proteins parallel with the maturation. The effect of *a deficiency of vitamin C* gives rise to a retrogression ("*dedifferentiation*") in the function of the mature dentin cells to one resembling the undifferentiated growing cells in the growth layer of the root of the tooth. Thus from the inert chromocentre of the mature dentin cell nucleus new ribose polynucleotide-containing nucleolar substance can be developed, after which ribose polynucleotides appear in the cytoplasm.

Thus it is probable that the decrease in the cellular growth activity, characteristic of the process of cell maturation and taking place in an early phase of the unipotent cell development is primarily caused by a reduced function of an unchanged amount of nucleolus-associated chromatin regulating the endocellular ribose polynucleotide-metabolism. This chromatin is not eliminated quantitatively until during later, more far-reaching differentiation-phases.

F. Summary.

A comparison of cytochemical and cytologica ldata during blood cell maturation shows: 1. that the decreasing growth activity of the individual cell during maturation is regulated by a definite part of the chromatin, during the interphase defined as the nucleolus-associated chromatin.

2. that the nucleolus-associated chromatin and the heterochromatic chromosome regions in Drosophila are functionally comparable conceptions.

3. that the nucleolus-associated chromatin proceeds direct from the gene-bearing chromosome material.

4. that the decrease in growth activity during the process of cell maturation is primarily caused by a reduced function of an unchanged amount of nucleolus-associated chromatin. Not until later, when the differentiation is far advanced, is this chromatin eliminated quantitatively.

PART II.

The Relationship of Specific Cell Differentiation Processes to the Growth of the Cell.

CHAPTER I.

Erythropoiesis.

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A. Introduction.

The development of tissues from the fertilized egg cell during which the blastomeres lose their omnipotential character and become unipotential is designated "differentiation". The same term is also used for the development of the unipotent cell from a lower to a higher stage of maturity and function. The latter form of *differentiation* is distinguished from the *growth* of the tissue cells.[1]

Similarly, during the development of the blood cell from the stem cell up to the mature cell capable of functioning, two cell-biological processes can be distinguished as essential, namely the *growth* or increase in cell mass and the differentiation, *i. e.* the formation of the specific cell-organ which makes the cell capable of performing its specific function.

As appears from what has been said before, the essential process in the *growth* of the tissue elements of the blood forming organ, the formation of the protein substances of the cell-body, is correlated with the metabolism of ribose polynucleotides within certain cell organellas.

[1] For literature concerned with these subjects, see the monographs by Fischer 1946, Hammet 1942, Weiss 1939, Wilton 1937 a. o.

From these studies it appears that the intensity of the growth processes during blood cell production is proportional to the amount nucleolar substance and the concentration of ribose polynucleotides in the cytoplasm. Inversely we hereby possess *the possibility of determining the growth conditions of the individual cell by means of analysis of the cellular concentration and distribution of the ribose polynucleotides.*

From the functional point of view the *differentiation* processes during the formation of the red blood cells are principally characterized by the formation of hemoglobin. If the hemoglobin content within each individual cell is measured during the different development phases of erythropoiesis, a measure is obtained of the degree of differentiation of the respective types of cells, expressed in a quantitative cytochemical unit.

If this can be done, the interplay between growth and the specific cell differentiation processes during the formation of the red blood corpuscles may be dealt with on a quantitative cytochemical basis.

B. Analyses.

The technique for the cytochemical analyses of hemoglobin is given in Chapter 3 (B, 2), Part I. The results of the analyses on normal bone marrow cells are accounted for in Chapter 4 (B, 2), Part I. These results are reviewed in table 17.

Table 17.

Hemoglobin analyses of normal erythropoietic cells.

Celldiameter μ	Hb%[1]	Total amount of Hb in 10^{-6} μg[2]
10.5	0	0
9.5	1	0.5
8.5	2	2
8.0	2	4
7.8	4	5.5
7.5	7	10
6.9	12	22
6.7	14	25
6.5	16	25
6.2	19	27
6.0	20	28
Mature red cells	30	27
»	32	28
»	33	28

[1] Measured at points in the cytoplasm.
[2] Obtained from integration over the cellular volume, cf p. 44 and fig. 23.

C. The Relationship of the Synthesis of Hemoglobin to the Growth Processes of the Blood Cell.

In the immature erythroid stem cell, which is characterized by high concentrations of ribose polynucleotides in the cytoplasm and nucleoli connected with an intensive cellular protein-formation, no trace of hemoglobin can be discovered. The function of the cells in this phase of development appears to have entirely to do with the growth processes. In the following developmental phase also ("the basophilic erythroblast"), where the concentration of ribose polynucleotides has decreased to about half its value in the previous cell type, the hemglobin concentration of the cytoplasm is hardly measurable (< 0.5 %). Not until after the stage of development ("the polychromatic erythroblast"), where the concentration of polynucleotides in the cytoplasm has fallen to below 0.5 %, and where the new-formation of cellular proteins has been practically completed, does the concentration of hemoglobin begin to increase to any extent worth mentioning. Subsequently the synthesis of hemoglobin is rapid; from this phase to the cell defined as "orthochromatic erythroblast" the concentration increases from 1 or 2 % to 20 %. During this period the cytoplasmic concentration of ribose polynucleotides has fallen to a point where it is not demonstrable.

If the hemoglobin concentration values are integrated over the whole cell volume, which is permissible in this case (see Chap. 2 (B, 2), Part I), the total amount of hemoglobin within each individual cell is obtained. The diagram in fig. 48 shows these values, together with the polynucleotide values.

The quantitative changes in the cell composition during the maturation phases show, in the first place, that before the beginning of the processes which lead to the hemoglobinization of the cell, the metabolisms which were shown above to be connected with the increase in the cell mass, have been practically completed. In the second place, it appears from the diagram that the increase in the total amount of hemoglobin per cell unit does not take place uniformly. The rate of the increase is at first relatively slow up to the phase of development which is characterized by a cell volume of 250 μ^3. At the same time the concentration of ribose polynucleotides in the cytoplasm has disappeared. Between the phases 250 — 150 μ^3 the rate of hemoglobin increase is greatest — from $1 \cdot 10^{-6} \mu g$ to $23 \cdot 10^{-6} \mu g$. It then stops at about a value of

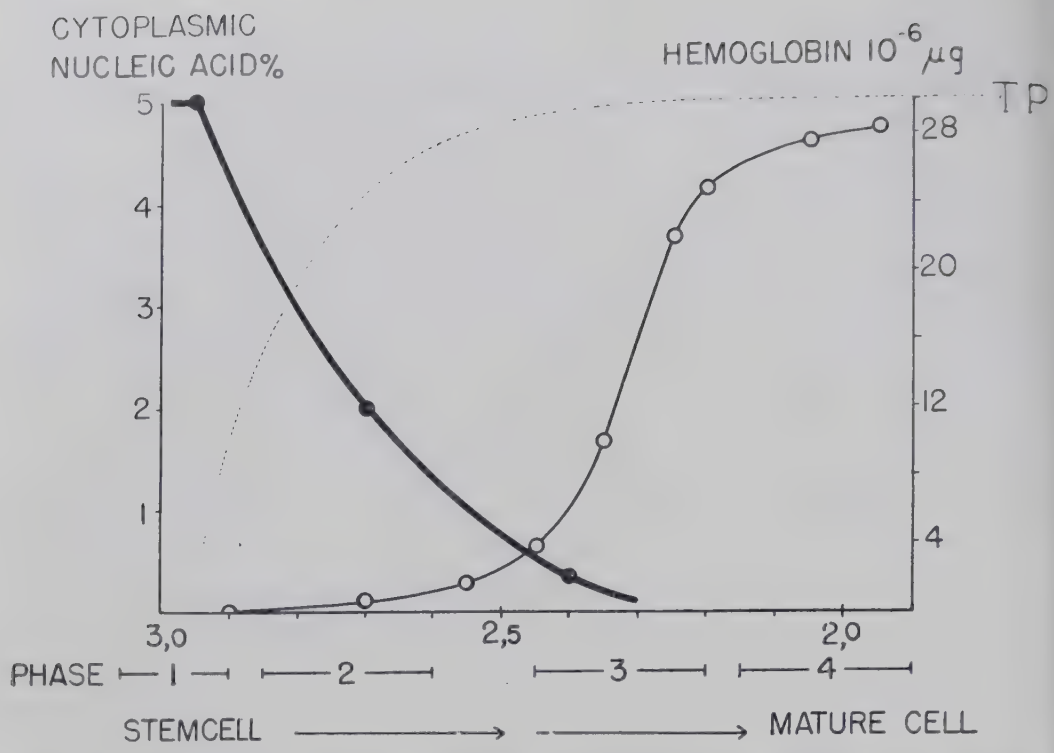

Fig. 48. Survey of the essential changes in cytoplasmic composition during erythropoiesis. Abscissa: logarithm of cellular volume (cf. fig. 44). —•—•— cytoplasmic ribose polynucleotides %, —○—○— total amount of hemoglobin in the cell, calculated from the absorption measurements in the Soret-band (cf. fig. 23 and 37). TP total cellular protein obtained from the total volume of cells in each development phase (see chap. 5, Part I). The figure shows on the whole, that before the erythroid cell is differentiated into its final functional stage, the ribose polynucleotide metabolism associated with the endocellular growth processes is finished, *i. e.* that during the unipotent cell development the new-formation of the cellular protein substances is completed *before* differentiation occurs into its final, specific form. For further details, see text.

$28 \cdot 10^{-6}$ µg, the same amount as an erythrocyte circulating in the blood stream may be calculated to contain.

Thus on quantitative cytochemical basis the unipotent red cell development occurs in several different phases, each of which is characterised by a particular cellular activity as regards its generation of cellular protein.

During the first phase, *the growth phase*, it is principally the formation of the basic cell protein substances that takes place. The cell then has a cytochemical organization resembling that of growing cells in general, *e. g.* embryonal cell. The next phase involves a *decline in the growth processes*, cytochemically observable as a rapid decrease in the concentration of the cytoplasmic ribose polynucleotides and the nucleolar mass. At the end of the decline in growth, the first signs of the processes which lead up to the differentiation of the cellular proteins into their final specific

forms begin to be observable. These processes become increasingly rapid, and at the phase where the concentration of cytoplasmic nucleotides reaches the zero value, a great generation of hemoglobin sets in — *the differentiation phase*. When the cell has developed to a mature stage, the formation of hemoglobin declines —, *the phase of declining differentiation*. The total amount of hemoglobin in the cell approaches a value of $28 \cdot 10^{-6}$ μg, corresponding to that of the finished erythrocyte. Thus, in the last stage with pyknotic nuclear degeneration and during the denucleation process to the erythrocyte of the blood, the hemoglobin content of the cell does not increase. The concentration becomes greater (from 25 to 33 %), owing to the decrease in volume during this process.

These results may throw light on some parts of the chemistry of the synthesis of hemoglobin during erythropoiesis. The proteid hemoglobin consists of two components, a Fe-containing prosthetic group of porphyrin type and coupled to it a basic protein substance, globin.

The formation of the main part of the globin component probably takes place principally in the same way as the new-formation of cellular proteins in general, *i. e.* with the cooperation of ribose polynucleotides. As their concentration in nucleoli and cytoplasm was greatest in the very earliest phases of maturation and then declined rapidly, the assumption that the synthesis of the globin material chiefly takes place during a comparatively early phase of erythropoiesis appears justifiable (see fig. 48, curve *TP*).

On the other hand, the formation of the Fe-porphyrin component and probably also the simultaneous coupling to hemoglobin takes place chiefly during a later maturation phase as shown in fig. 48.

Thus the cytochemical analysis data above render it probable that the synthesis of hemoglobin during erythropoiesis is effected during two phases separate in time, the first involving the formation of the protein component during the cellular growth phase, the second involving the formation of the prosthetic group and the coupling to the complex hemoglobin during a later differentiation phase.

D. Summary.

The analyses in Part I of the endocellular growth processes afford a possibility of investigating on a quantitative cytochemical

basis the interplay between the growth of the blood cells and the differentiation of the cellular proteins into their specific functionating forms. The main cellular protein synthesis (growth) takes place during the earliest development stage and ribose polynucleotides are involved in this process. The subsequent development phase is characterized by a cessation of cellular growth activity, paralleled by a rapid decrease in cytoplasmic nucleotide concentration (5 % to < 0.5 %). After this phase the main synthesis of hemoglobin begins, and the cellular content rises rapidly from an amount less than $2 \cdot 10^{-6}$ μg up to $25 \cdot 10^{-6}$ μg, the final value being $28 \cdot 10^{-6}$ μg. Owing to diminishing volume, the hemoglobin concentration increases from 25 % to 33 % in the circulating erythrocyte. Thus on a quantitative cytochemical basis the unipotent stem cell development normally occurs in several different phases, each of which is characterised by a particular cellular activity: 1. the phase of growth, 2. the phase of declining growth, 3. the differentiation phase, and finally, 4. the phase of declining differentiation.

CHAPTER 2.

Growth and Differentiation Processes During the Formation of Other Tissue Cells.

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In this chapter an investigation will be made into, whether the above-shown scheme of the course of the growth and differentiation processes for production of cellular proteins during erythropoiesis, can be applied in principle to other tissue cells.

A. The Growth and Differentiation of Dentine Cells.

The regular topography of the dentine tissue in the incisors of rodents renders it possible to survey cell development in the form of growth and maturation (see WILTON 1931, 1937).

The metabolic processes of polynucleotides in the dentine cells of guinea pigs, correlated with the growth of the cell, has been microspectrographically analysed by THORELL and WILTON (1945). The analyses show that, during the maturation of the dentine cells, in the case of an occlusal shift from the growth zone of the root, a continuous decline in the function of the mechanism for the new-formation of cytoplasmic proteins took place (see lit. cit.).

The ribose polynucleotide-containing nucleolar mass and the high concentrations of cytoplasmic nucleotides, which characterized the growing cells in the root zone, disappeared in the mature dentine cells. Microspectrographically only cytoplasmic proteins and in the nucleus inactive — from the point of view of the protein new-formation — Feulgen-positive chromocentra could be demonstrated.

The process which constitutes the basis of the differentiation of the dentine cells for their specific function as hard tissue, is mainly the deposition of Ca-salts, conditioned by specific cytoplasmic protein substances, such as phosphatases (see ROBISON et al. 1923—1934, GOMORI 1943).

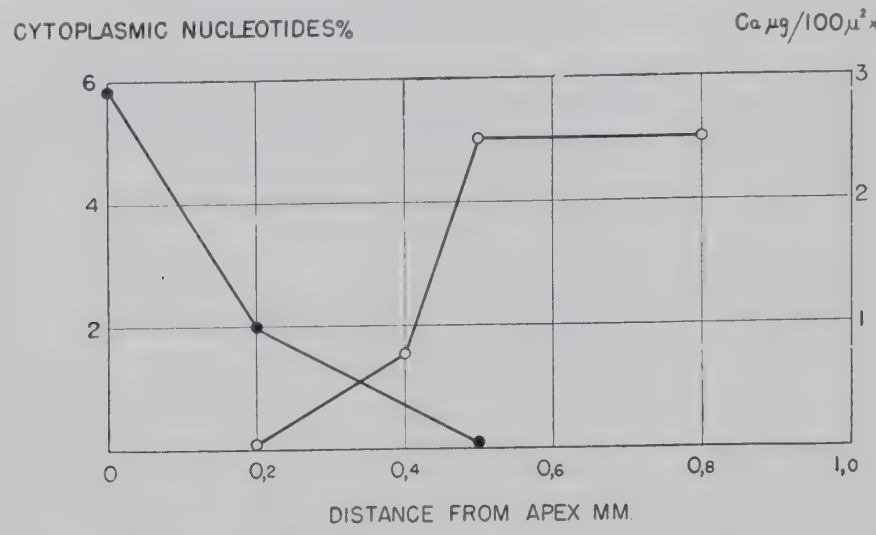


Fig. 49. Analysis of the cytoplasmic ribose polynucleotide concentration and the cellular calcium content in the dentine from the tip of the root to the occlusal part in a microtome section of a guinea pig's tooth. See text.

The Ca-contents in guinea pig incisor dentine cells during the various phases of maturation were analysed by X-ray microspectrography, according to ENGSTRÖM 1946 (see lit. cit.). The data from the polynucleotide analyses and Ca-analyses respectively are compared in fig. 49. The cytochemical changes which form the basis of growth and differentiation during the development of the dentine cells agree quantitatively with those discussed above. The cells undergo a growth phase in the root zone characterized by an endocellular ribose polynucleotide mechanism as described earlier, followed by a phase of declining growth. During this phase the first signs of Ca-deposits appear. When the cells are quite inactive from the point of view of growth — the zero value for the cytoplasmic polynucleotides — the differentiation phase begins with a rapid increase in the Ca-content. After this an equilibrium in the differentiation is reached, when the Ca-content remains constant.

Thus the analysis data from the dentine cells in the incisors of guinea-pigs support what was shown in the preceding chapter to apply to the growth and maturation processes of erythropoiesis.

This indicates that these principles for the processes of the development of the unipotent stem cell into a functioning tissue cell are of a general character.

B. Granulocytopoiesis.

It has been shown above that, for granulocytopoiesis, the most immature cells have an intensive metabolism of ribose polynucleotides associated with growth, which gives the cells in this phase of development their characteristics. During the subsequent maturation, the polynucleotides in the cytoplasm and the ribose polynucleotide-containing nucleolar mass disappear continuously, parallel with decreasing growth activity of the individual cell, *i. e.* a similar course for the endocellular growth processes as during erythropoiesis.

The processes which are most closely connected with the differentiation of the cytoplasmic proteins in the granulocytes are the formation of the granula. The granula probably constitutes the basis of the principal function of the mature white blood corpuscles, the de-toxication of bacterial toxins (AGNER 1947).

The phase of the appearance of granula and of the formation of granula during granulocytopoiesis has been the subject of exhaustive morphological investigations. The granula formation does not start until the promyelocyte phase and seems to occur most rapid during the myelocyte phase (see for example SABIN 1928). According to the analyses in Part I no cytoplasmic ribose-polynucleotides can be detected in the myelocyte.

Thus, the data obtained do not gainsay the assumption that conditions similar in principle to those prevailing during the erythropoiesis are present here (see Chap. 1). Since, as has been shown above, the development of the dentine cells takes place according to these principles, the probability is thereby increased that it is also the case during granulocytopoiesis.[1]

C. Cancer Cells.

In this connection it will be of interest to compare the growth and differentiation processes which normally take place during

[1] AGNER (1941) detected verdoperoxidase in certain blood cells. It is probable that verdoperoxidase is an essential part of granula in the granulocytes. As the extinction coefficient for 1 % VPO cm in the Soret band is c. 100, this gives the measurable extinction of 0.1 for a 10 μ layer. According to AGNER, verdoperoxidase is present intracellularly in the granulocytes in a total concentration of up to 5 %, and therefore the new formation of it during granulocytopoiesis can be quantitatively analysed by the method indicated in Chap. 3.

the maturation of the blood cells and dentine cells, with the changes ("differentiation") of the malignant growing cells in the different layers of a cancer cord (CASPERSSON and SANTESSON, 1942). These authors have shown it to be probable that in epithelial cancers the malignant growth is due to a pathological disturbance in the gene parts which normally regulate the growth of the cell. Cytochemically the disturbance is observed as an intensive hyperfunction of the cellular mechanism of the nucleolar-cytoplasmic polynucleotides. According to CASPERSSON and SANTESSON, the characteristic changes from an infiltrative growing cell in the peripheral layer (A-type) to an inner cell (B-type) on the way to necrosis is caused by the poor nutrition in the centre of the cancer cord.

The nucleoli of the non-growing B-cell type are large and still contain considerable amounts of ribose polynucleotides. On the other hand, during the normal maturation of the blood cells, nucleolar development is suppressed parallel with the decrease in the intensity of growth. The difference is apparent: in the former case of development from a growing A-cell to a necrotizing B-cell the gene mechanism regulating growth is abnormally active the whole time, and the cytochemical changes are caused primarily by extracellular factors. During the maturation of the blood cell, the decline in the intensity of growth is primarily due to the nuclear centres regulating the cellular formation of protein, the nucleolus-associated chromatin.

D. Summary.

The principles which were shown in the preceding chapter to apply to the growth and differentiation processes of the unipotent blood stem cell into the mature red blood corpuscle is shown by analysis data from the literature to apply also to the production of the dentine cells in the incisors of the guinea pig. In all probability there are also similar conditions during granulocytopoiesis. On the other hand, from the cytochemical point of view ,the "differentiation" for example of the cells in the different parts of a cancer cord growing infiltratively have nothing in common with these principles for normal formation and differentiation of cellular substances.

PART III.

Cytochemical Analyses of the Formation of Blood Cells in Certain Pathological Conditions.

The analyses in Part III aim at investigating whether, on the basis of the results presented in Parts I and II, it is possible to find disturbances in the cell-physiological processes of growth and differentiation during certain pathological conditions in blood cell formation.

CHAPTER I.

Disturbances of Growth in the Nucleated Blood Cells.

An investigation will be presented in Chapter I as to whether any cytochemical effects can be observed in the case of pathologically disturbed growth. The material selected comprises certain severe growth hypertrophies of the nucleated blood cells: myeloid and lymphatic leukemias.

Case 1

Male, 60 years of age. The patient became ill in March 1946. Admitted to hospital $^{8}/_{5}$ 1946. Hb 80 %, red blood corpuscles 4.0 mill., white cells 214,000/mm³, of which 96 % resembled myeloblasts. The sternal puncture was abundant in cells which were almost exclusively immature, myeloid elements with strongly basophilic cytoplasm, chromatin-rich nucleus and distinct, acidophilic nucleoli. The liver and spleen were enlarged and palpable. Treatment with X-rays and blood transfusions, but in spite of this, increasing anemia. Died on $^{16}/_{7}$ 1946.

Pathological-anatomical diagnosis: *Leukemia myeloica acuta.*

Sternal puncture for cytochemical analysis on $^{11}/_{5}$ 1946. From the puncture preparations were made of living cells according to the method described in Part I, p. 46.

Cytochemical examination: The preparation consists exclusively of spherical cells of varying sizes. The cytoplasm is small, homogeneous and strongly ultraviolet absorbing (fig. 50). The nucleus is relatively large, with folded nuclear membrane. Apart from grains of chromatin of varying sizes, the nucleus contains a number of irregular, very strongly ultraviolet absorbing nucleoli.

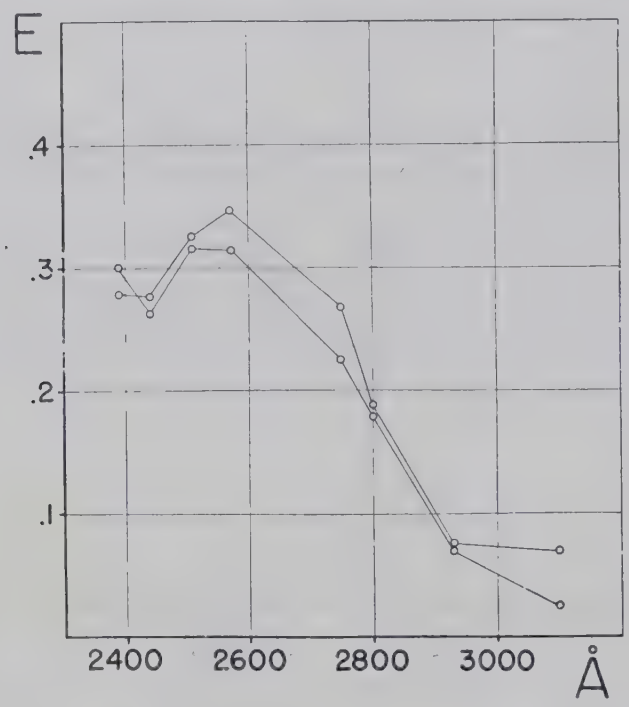


Fig. 51. Absorption spectra from points in the cytoplasm of bone marrow cells from *Leukemia myeloica acuta* (case 1).

After Feulgen's nucleal reaction there are observed in the nucleus a number of small Feulgen-positive aggregations of chromatin and distinct cavities, corresponding to the sites of the nucleoli, each surrounded by a disrupted superficial layer of ribodesose polynucleotide-containing nucleolus-associated chromatin. The cytoplasm is completely Feulgen-negative.

Fig. 51 shows the ultraviolet absorption spectra from points in the cytoplasm. These spectra may be regarded as representative mean values. In all the curves a strong polynucleotide maximum appears at 2600 Å. The mean value for a large number of determinations of the quotient between the extinctions at 2570 and 2750 Å is 1.24 (table 18). The relation between polynucleotides and protein in the cytoplasm may be calculated to be as high as about 1 : 4 with a polynucleotide concentration up to 5 %. The measurement values show that the polynucleotide concentration in the cyto-

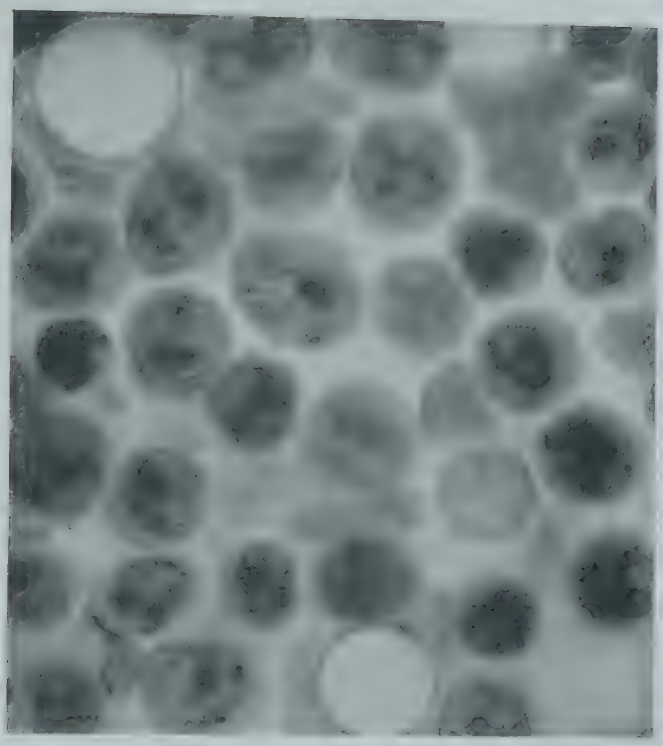


Fig. 50. Microphotographs taken at 2570 Å of living bone marrow cells from *Leukemia myeloica acuta* (Case 1). 1500 ×.

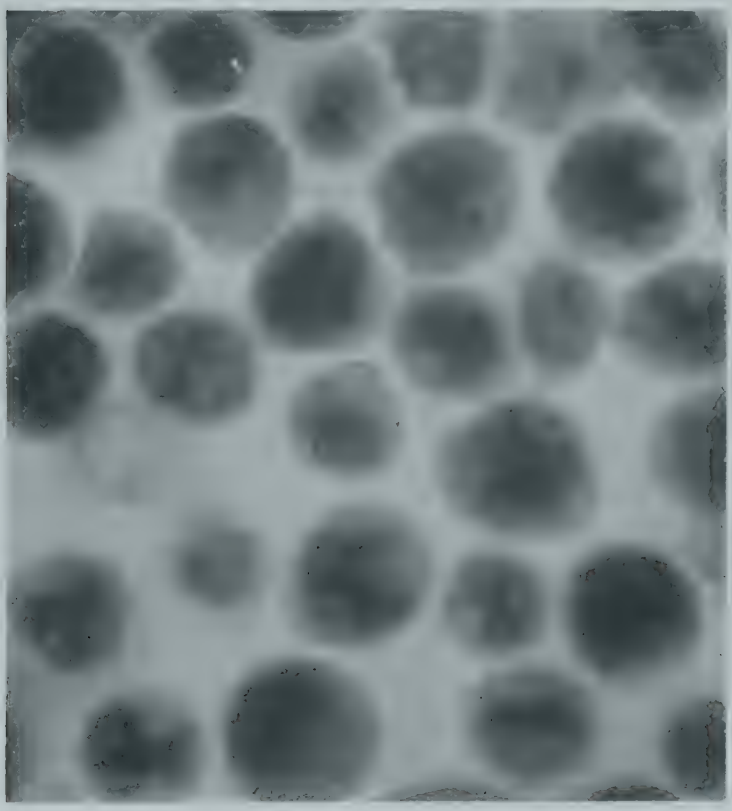


Fig. 52. Microphotographs taken at 2570 Å of living bone marrow cells from *Leukemia myeloica acuta* (case 2). 1500 ×.

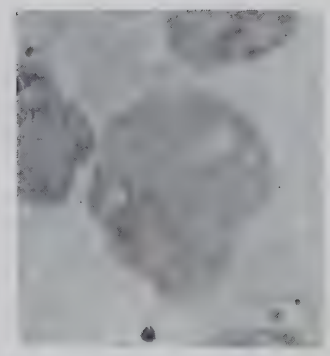


Fig. 53. Feulgen-stained bone marrow cell from *Leukemia myeloica acuta* (case 2). Note the Feulgen negative nucleoli and the surrounding nucleolus-associated chromatin. 2000 ×.

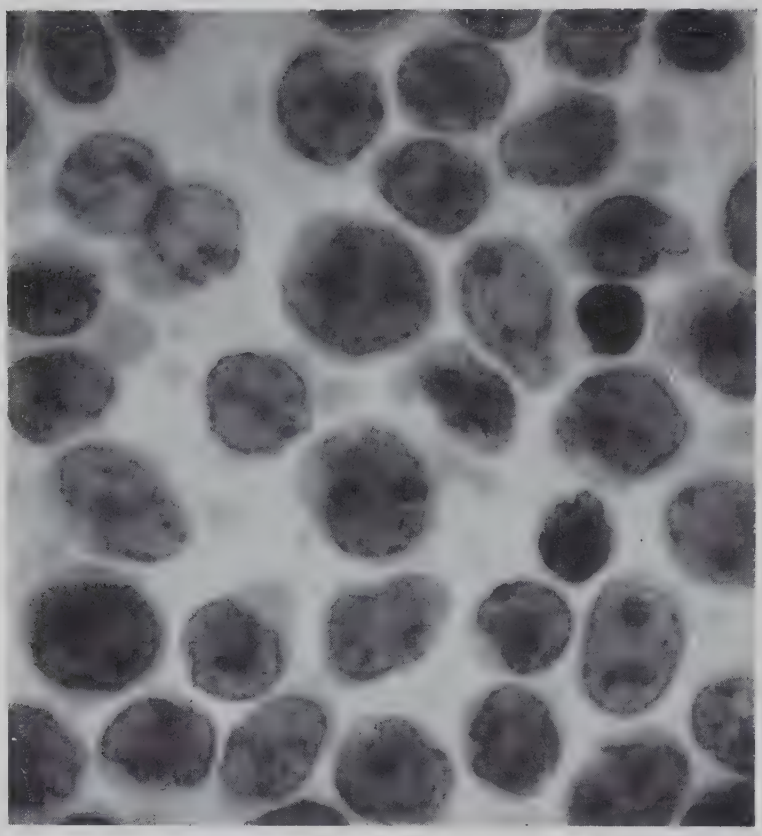


Fig. 54. Living lymph gland cells from *Leukemia lymphatica* photographed at 2570 Å. 1500 ×.

plasm varies considerably, from 3 % up to 5 % from one cell to another, but the values are fairly high throughout. The nucleoli in these cells are too deeply embedded in highly absorbing chromatin for the determination of their ultraviolet absorption.

Table 18.

The quotient $\frac{E_{257}}{E_{275}}$ *for points in the cytoplasm of bone marrow cells from* ***Leukemia myeloica acuta*** (case 1).

Cell	E_{257}	E_{275}	Q
1.....	0.220	0.165	1.33
2.....	0.250	0.200	1.25
3.....	0.284	0.230	1.24
4.....	0.280	0.235	1.18
5.....	0.305	0.240	1.27
6.....	0.290	0.230	1.26
7.....	0.452	0.360	1.26
8.....	0.420	0.350	1.20
9.....	0.190	0.165	1.15
11.....	0.346	0.284	1.22
10.....	0.337	0.260	1.29
12.....	0.212	0.170	1.25

$M = 1.24 \pm 0.01$

Case 2

Male, 34 years of age. The patient became ill on $^{30}/_{10}$ 1946. Admitted to hospital $^{1}/_{11}$ 1946. Hb 80 %, red blood corpuscles 3.7 mill., white 4,000, of which 45 % resembled myeloblasts, 50 % lymphocytes and 5 % granulocytes. The sternal puncture was poor in cells, with myelopoiesis suppressed after the promyelocytic stage. At the beginning of December, a rapid increase of white blood cells in the blood began, reaching 303,000, of which 80 % resembled myeloblasts. Increasing anemia (Hb < 50 %), in spite of numerous blood transfusions. Sternal puncture on $^{7}/_{12}$: Abundant in cells, the picture was dominated by a strongly hyperplastic reticulum with differentiation towards myeloid cells. Complete absence of mature cells. Great deterioration of the general condition and death on $^{3}/_{1}$ 1947.

Diagnosis: *Leukemia myeloica acuta.*

Sternal puncture for cytochemical analysis on $^{7}/_{12}$ 1946. The living bone-marrow cells were mixed with physiological saline solution, after which a preparation on quartz slide was made.

Cytochemical examination: The picture of the living cells in the ultraviolet microscope at 2570 Å is strikingly uniform (fig. 52). The cells are large, spherical and with a high nucleus-plasma ratio. Optically the cytoplasm is homogeneous and strongly ultraviolet

absorbing at 2570 Å. The nucleus has a folded nuclear membrane and, apart from grains of chromatin of varying sizes, contains 2 to 4 large, strongly ultraviolet-absorbing nuclear bodies. Numerous mitoses can be observed.

After Feulgen's nucleal reaction, the folded nuclear membrane stands out as does also the Feulgen-positive nucleolus-associated chromatin surrounding the nucleoli (fig. 53).

Table 19.

The quotient $\frac{E_{257}}{E_{275}}$ *for points in the cytoplasm of bone marrow cells from Leukemia myeloica acuta* (case 2).

Cell	E_{257}	E_{275}	Q
1.....	0.500	0.380	1.31
2.....	0.480	0.380	1.26
3.....	0.440	0.320	1.37
4.....	0.620	0.480	1.29
5.....	0.360	0.300	1.20
6.....	0.405	0.310	1.30
7.....	0.512	0.385	1.33
8.....	0.542	0.410	1.32
9.....	0.602	0.465	1.30
10.....	0.302	0.232	1.30
11.....	0.443	0.330	1.34
12.....	0.394	0.318	1.25
13.....	0.560	0.425	1.31
14.....	0.522	0.390	1.33

$M = 1.30 \pm 0.01$

Determination of the ultraviolet absorption of points in the cytoplasm at the absorption maximum of polynucleotides gives a high extinction, between 0.5 and 0.6. The quotients between the extinctions at 2570 and 2750 Å are high, on an average 1.30 (table 19). Analytical data show that, in the cytoplasm and the nucleolar apparatus, there are considerable amounts of ribose polynucleotides. From the measurement data the latter can be calculated in the cytoplasm to reach concentrations between 5 and 7 %.

Case 3

Girl, 16 years of age. Became ill in September 1944. Admitted to hospital on $^{24}/_{10}$ 1944. Enlarged and palpable glands on the neck and in the axillae. The spleen was greatly enlarged. Hb 55 %, red blood corpuscles 3.0 mill/mm^3, white cells 3,000, of which 40 % were granulated and 60 % were lymphocytes at different stages of maturation. The sternal puncture was abundant in cells and consisting exclusively of elements resembling lymphoblasts. Repeated blood transfusions. In

February 1945 the spleen could be palpated below and to the right of the navel. Blood: Hb 20 %, red blood corpuscles 1.1 mill/mm³, white cells 20,000, of which 10 % were granulated, the remainder being immature lymphoid elements. Died on $^{18}/_{3}$ 1945. At the autopsy the spleen weighed 2,300 g.

Pathological-anatomical diagnosis: *Leukemia lymphatica.*

On $^{7}/_{12}$ 1944 a jugular gland was excised for cytochemical analysis. A preparation of living cells in physiological saline solution was made on quartz slide as above.

Cytochemical examination: Examination in the ultraviolet microscope at 2570 Å reveals a uniform picture of relatively small cells of irregular shape and high nucleus-plasma ratio (fig. 54). The cytoplasm is homogeneous and strongly ultraviolet-absorbing, except for a small, less strongly absorbing area at a concavity in the nucleus.

The nucleus is irregular in shape, abundant in chromatin and has a folded nuclear membrane. The nucleus contains 2 to 4 large, strongly ultraviolet-absorbing nucleoli.

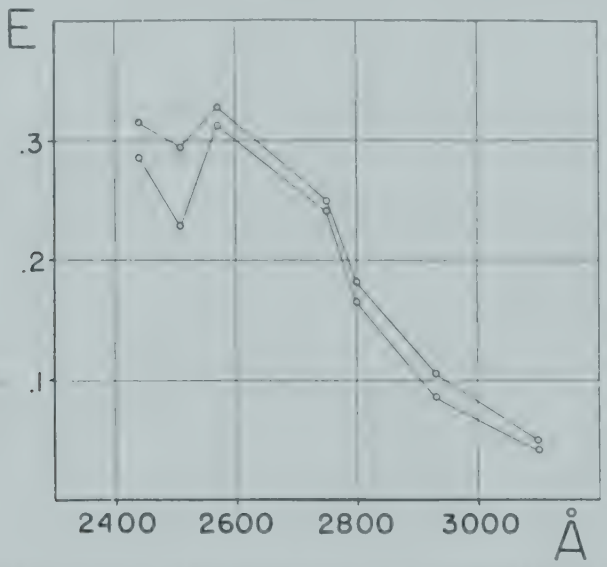


Fig. 55. Absorption spectra of points in the cytoplasm of lymph gland cells from *Leukemia lymphatica.*

After Feulgen's nucleal reaction the disrupted nucleolus-associated chromatin surrounding the nucleoli stands out distinctly. The cytoplasm is entirely Feulgen-negative.

The ultraviolet absorption curves from points in the cytoplasm show a marked polynucleotide band at 2600 Å (fig. 55). From the absorption data the polynucleotide concentration of the cytoplasm can be calculated to be 5 %—7 %.

Survey of the Results of Chapter I.

The disturbances in growth of the nucleated blood cells which were examined above are all characterized by an intensive growth hypertrophy of a definite cell type. The analyses showed that the cytochemical picture is dominated throughout by large concentrations of ribose polynucleotides. The intensive pathological growth of these myeloid and lymphatic cell elements takes place in the presence of large quantities of ribose polynucleotides in the cytoplasm and the nucleolar apparatus. The measured concentrations of cytoplasmic polynucleotides in the pathologically growing cells are equal to, or exceed, those in the stages of most intensive growth during normal hematopoiesis.

If a comparison is made between the polynucleotide metabolism of the pathologically growing cells and that of the normal hematopoiesis an obvious difference appears. In the latter case, the cells after their growth phase immediately undergo a rapid decline in their ribose polynucleotide metabolism (see Chap. 5, Part I). In no case in the pathologically intensively growing cells can this decline in protein formation intensity be found. Instead, in the blood forming organ, the cells accumulate with very high concentrations of ribose polynucleotides in the cytoplasm and nucleolar apparatus. With the present methods it is impossible to discover what the difference between the normally strongly growing myeloblasts and these pathologically growing cells (observable in the first place as polymorphism, strongly folded nuclear membrane and an irregular formation of nucleoli) involves cytochemically. However, it is clear that the endocellular metabolism of these pathologically growing cells may be characterized as being completely centred on the new-formation of cellular proteins, during which process the cell *exaggerates and makes abnormal use* of *the ribose polynucleotide metabolism normally functioning during the growth phase of blood cell renewal.*

Thus the cytochemical effects in the case of these pathological blood cell formations are of such an order of magnitude that, with the methods of analyses employed, they render possible a measurable distinction between the normal and pathological courses of blood cell formation.

Furthermore, it appears from the results that, in the case of these pathologically increased endocellular concentrations of

ribose polynucleotides, the effects are so great (> 5 %), that they permit gradation. If this is compared with the conclusions above (Chap 5, Part I), that the growth conditions of the individual cell can be judged by means of an analysis of the ribose polynucleotide concentration and distribution in the cell, this appears to imply a possibility of grading the pathological growth disturbance on the basis of quantitative cytochemical analysis data.

CHAPTER 2.

Disturbances in the Erythropoiesis.

Erythropoiesis was found to be suitable for investigations of the relationship between cellular growth and the differentiation of cellular substances, since the essential process during the maturation of the red blood cells, the synthesis of hemoglobin, could be followed relatively easily with quantitative cytochemical methods of analysis (see Part I and II). The analyses showed that the normal development of the unipotent stem cell into mature erythrocytes could be divided into phases, each of which was characterized by its special cytochemical activity. Thus, *before* the endocellular synthesis of the hemoglobin-complex occurs, the bulk of the endocellular ribose polynucleotide metabolism associated with growth is finished.

In this chapter will be described an investigation of changes which may occur in the normal processes in the case of pathological disturbances in the formation of red blood cells, such as anemias. Examples selected were pernicious and hemorrhagic anemia.

A. Pernicious Anemia.

Case 1

Male, 60 years of age. The patient was admitted to the hospital on $^{27}/_{5}$ 1946 with a typical picture of pernicious anemia. Hb 35 %, red blood corpuscles 1.4 mill. and white cells 2,500 per mm³. Anisocytosis and macrocytosis. The sternal puncture was abundant in cells, mainly megaloblasts. He reacted promptly to liver injections with reticulocytosis and subsequent increase of Hb and red cells. He was discharged as improved on $^{5}/_{7}$ with Hb 82 % and red blood cells 4.45 mill./mm³.

Sternal puncture for cytochemical analysis on $^{28}/_{5}$ 1946. Preparations of living cells in physiological saline solution were made on quartz slide.

Cytochemical examination: The preparation is abundant in cells. An examination in the ultraviolet microscope at wave length

2570 Å shows most of the cells resemble megaloblasts of varying sizes. These cells are round or oval and have a relatively high nucleus-plasma ratio. The cytoplasm is homogeneous optically and absorbs ultraviolet light strongly. The nucleus is regular in shape, rich in chromatin and contains one or more large, strongly absorbing nucleoli (fig. 56).

After Feulgen's nucleal reaction the ribodesose polynucleotide-containing chromatin stands out, as do small distinct cavities corresponding to the nucleoli, surrounded by nucleolus-associated chromatin. The cytoplasm is completely Feulgen-negative.

Photographic-photometrical determinations of the ultraviolet light absorption of points in the cytoplasm were made for a number of these cells. Subsequently the preparation was subjected to microspectrography in the visible part of spectrum. By using a system of micro-coordinates according to Maltwood, the light absorption in Hg 4358 Å and 4960 Å could be determined in the identical cells which were examined in ultraviolet (fig. 57). For analytical values see table 20.

Table 20.

Ribose polynucleotide and hemoglobin analyses of points in the cytoplasm of bone marrow cells from pernicious anemia (case 1).

Celldiameter μ	E_{257}	E_{275}	E_{435}	E_{496}	$Q\frac{E_{257}}{E_{275}}$	RNA%	Hb%
10	0.132	0.110	0.020	0.010	1.20	4	0
8	0.155	0.125	0.100	0.010	1.24	5	14
7.5	0.150	0.120	0.130	0.015	1.25	5	18

The results show that these megaloblast-like cells contain hemoglobin in their cytoplasm, the concentration varying apparently with the size of the cell. This is analogous with the conditions occuring during normal erythropoiesis. Thus in the very largest cells no porphyrin absorption can be detected, while in the smaller ones the hemoglobin concentration can be calculated from the light absorption data to attain values as high as 18 %. From the light absorption in ultraviolet the cytoplasm in all these cells can be calculated to contain between 4 and 5 % of polynucleotides. As the cytoplasm was completely Feulgen-negative, the polynucleotides must be of the ribose type.

Case 2

Female, 56 years of age. The patient was admitted to the hospital in April 1946 with typical symptoms of pernicious anemia. Hb 42 %, red blood cells 2 mill. and white blood cells 3,000/mm³, macrocytosis and anisocytosis. Sternal puncture abundant in cells, mainly megaloblasts. She was treated with liver preparations and discharged as improved on $^{30}/_{4}$ 1946 with 70 % Hb, 3.5 mill. red blood cells and 4,200 white cells/mm³.

On $^{3}/_{4}$ 1946 sternal puncture was performed for cytochemical analysis of her bone marrow cells.

Examination of the living cells in the ultraviolet microscope at 2570 Å shows that the preparation consists chiefly of large, megaloblast-like elements. The cytoplasm is homogeneous and strongly ultraviolet absorbing. The nucleus is relatively large, of regular shape and, apart from grains of chromatin of varying sizes, contains one or a couple of large, strongly absorbing nucleoli.

After Feulgen's nucleal reaction only the nuclear structures develop colour. The nucleolus-associated chromatin surrounding the nucleolar cavities stands out particularly clearly.

Table 21.

Ribose polynucleotide and hemoglobin analyses of points in the cytoplasm of bone marrow cells from pernicious anemia (case 2).

Celldiameter μ	E_{257}	E_{275}	E_{435}	E_{496}	$Q\frac{E_{257}}{E_{275}}$	RNA%	Hb%
10	0.216	0.173	0.015	0.010	1.25	5	0
9	0.253	0.205	0.180	0.065	1.24	5	15
7	0.195	0.163	0.100	0.020	1.20	4	15
7	0.173	0.140	0.140	0.015	1.24	5	20
7	0.113	0.098	0.108	—	1.16	3	15

Quantitative determinations of the light absorption of points in the cytoplasm both in the visible and the ultraviolet spectral ranges were made in identical cells as in the preceding case. For values see table 21. Here, too, the cytoplasm was shown to contain varying concentrations of hemoglobin according to the size of the cell, from non-detectable quantities up to 20 %. On the other hand, the absorption analyses in ultraviolet show a fairly constant cytoplasmic polynucleotide concentration of between 4 and 5 %.

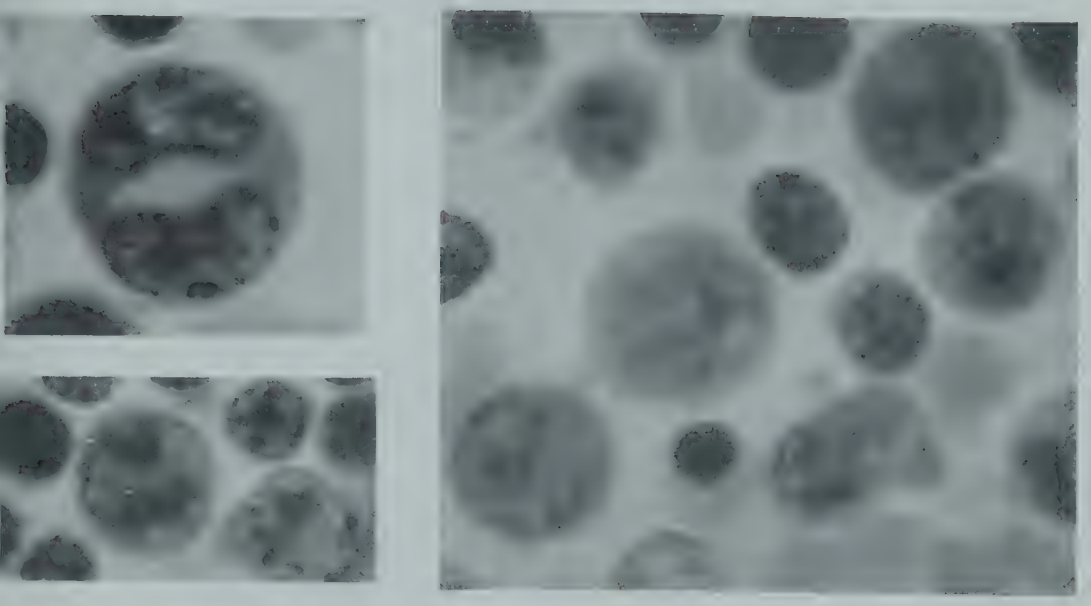
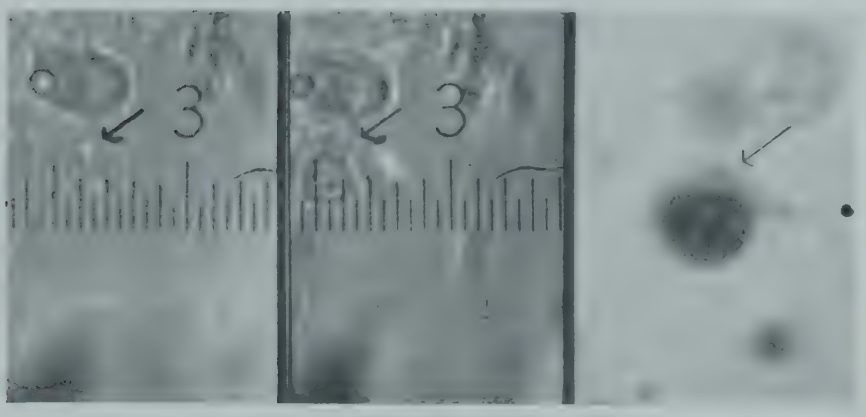
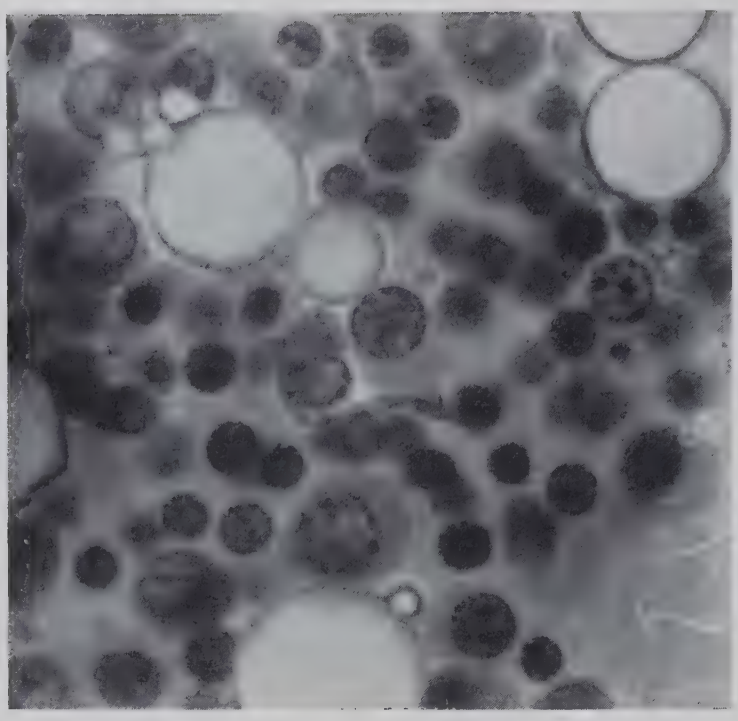


Fig. 56. Living bone marrow cells from *Pernicious anemia* (case 1) photographed at 2570 Å. 1500 ×.

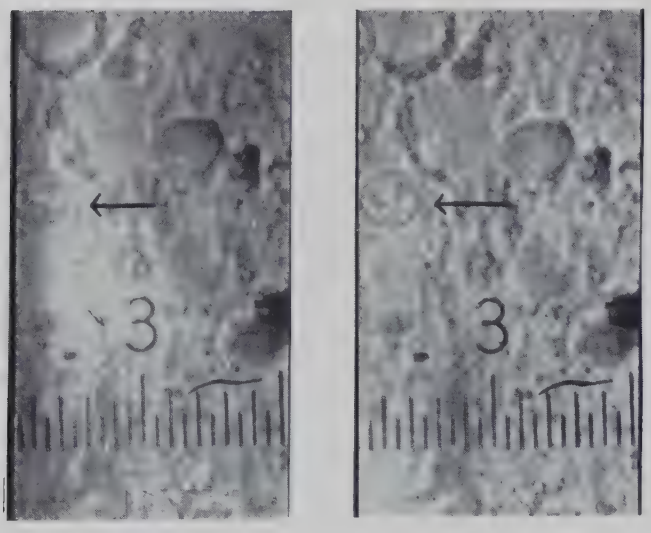


A B C

Fig. 57. Living bone marrow cell (megaloblast) from *Pernicious anemia* photographed at Hg 496 mμ (A), Hg 435 mμ (B) and Cd 257 mμ. Note the high cytoplasmic absorption in the Soret-band (B) and at 257 mμ.



A



496 mμ 435 mμ

B

Fig. 58. A is a microphotograph taken at 2570 Å of living bone marrow cells from *Hemorrhagic anemia*. 1000 ×. Large amounts of erythroblasts in different maturation stages.
B shows an erythroblast (indicated by the arrow) from *Hemorrhagic anemia* photographed at Hg 496 mμ and Hg 435 mμ. The cytoplasm has no absorption neither in the long wave length nor in the Soret-band.

B. Hemorrhagic Anemia.

Rabbits weighing between 5 and 7 kilograms were used for investigations of this type of anemia. Between 40 and 80 cm^3 of blood were withdrawn every other day through the veins of the ears. After 10 days' tapping the amount of hemoglobin in the blood had fallen from 15 g/100 ml to 5 g/100 ml. The number of red blood cells decreased at the same time from 5 to 3 mill./mm^3. These values were kept constant by means of further tapping. On the 20th day, the animals were killed, and bone marrow specimens were taken from the femurs for cytochemical analysis.

Preparations of living bone-marrow cells were made on quartz slides as described above. Examination in the ultraviolet microscope at wave length 2570 Å shows that the bone marrow consists mainly of cells in all stages of erythropoietic development (fig. 58 a). On the whole the ultraviolet cytology of these cells does not differ from that of the cells of normal erythropoiesis (see Part I). Thus the largest have a high nucleus-plasma ratio, a homogeneous and strongly ultraviolet absorbing cytoplasm, and one or more large ultraviolet absorbing nucleoli in the nucleus. All stages of development between these and more mature cells with pyknotic, compact ultraviolet absorbing nuclei and slightly absorbing cytoplasm can be observed.

After Feulgen's nucleal reaction only the very largest cells are found to contain nucleal-negative nucleoli, surrounded by a thick superficial layer of nucleolus-associated chromatin. The cytoplasm does not develop colour.

Photographic-photometrical determinations of the absorption in ultraviolet of points in the cytoplasm show that, as in the cells of normal erythropoiesis, only the largest, most immature cell types contain cytoplasmic polynucleotides, attaining concentrations between 4 and 5 % (see table 22). In the subsequent phases of development the polynucleotides fall to non-detectable concentrations.

From the determinations of the cytoplasmic light absorption in the visible spectral range, according to the method indicated in Chap. 3, Part I, no substances of the porphyrin-absorption type can be found in the cytoplasm of the largest, most immature cell forms. For values see table 22. Analyses of the smaller cells, which, owing to their lack of cytoplasmic polynucleotides and to nuclear de-

generation in the direction of pyknosis, may be assumed to be more mature (cf. Part II), also show very low porphyrin values however; in no case do these exceed the values corresponding to 6—10 % of hemoglobin.

Table 22.

Ribose polynucleotide and hemoglobin analyses of points in the cytoplasm of bone marrow cells from hemorrhagic anemia.

Celldiameter μ	E_{257}	E_{275}	E_{435}	E_{496}	$Q\frac{E_{257}}{E_{275}}$	RNA%	Hb%
10	0.100	0.085	0.005	0.005	1.18	4	0
8	0.120	0.110	0.010	0.015	1.10	2	0
7	—	—	0.055	0.020	—	—	3
6	0.096	0.098	0.050	0.020	0.98	0	6
6	—	—	0.080	0.025	—	—	10
5.5	0.062	0.065	—	—	0.95	0	—

Survey of the Results in Chapter 2.

The changes in the processes of endocellular growth and differentiation, as shown by the results of the analyses of pernicious and hemorrhagic anemia respectively, are most easily displayed by inserting the values obtained in a system of coordinates similar to that which was used for the interpretation of the cytochemical analysis data of normal erythropoiesis. The diagrams in fig. 59 were obtained in that way.

During normal erythropoiesis there is a definite relationship between the growth and differentiation processes of the cellular substances (see Part II). Both the cases of *pernicious anemia* investigated show a clear and identical disturbance in this relationship (see fig. 59). The process of formation of cellular substances in these cases is clearly characterized by the *non-disappearance* of the ribose polynucleotides of the cytoplasm and nucleolar apparatus. Normally the ribose polynucleotides disappear during erythropoiesis and this begins during an early phase of maturation. On the other hand, formation of hemoglobin seems to take place in the bone-marrow cells in pernicious anemia. Thus in this disease of the blood-forming organ there are cells enriched with comparatively high concentrations of cytoplasmic polynucleotides; in some of these cells, the synthesis of hemoglobin has proceeded relatively far.

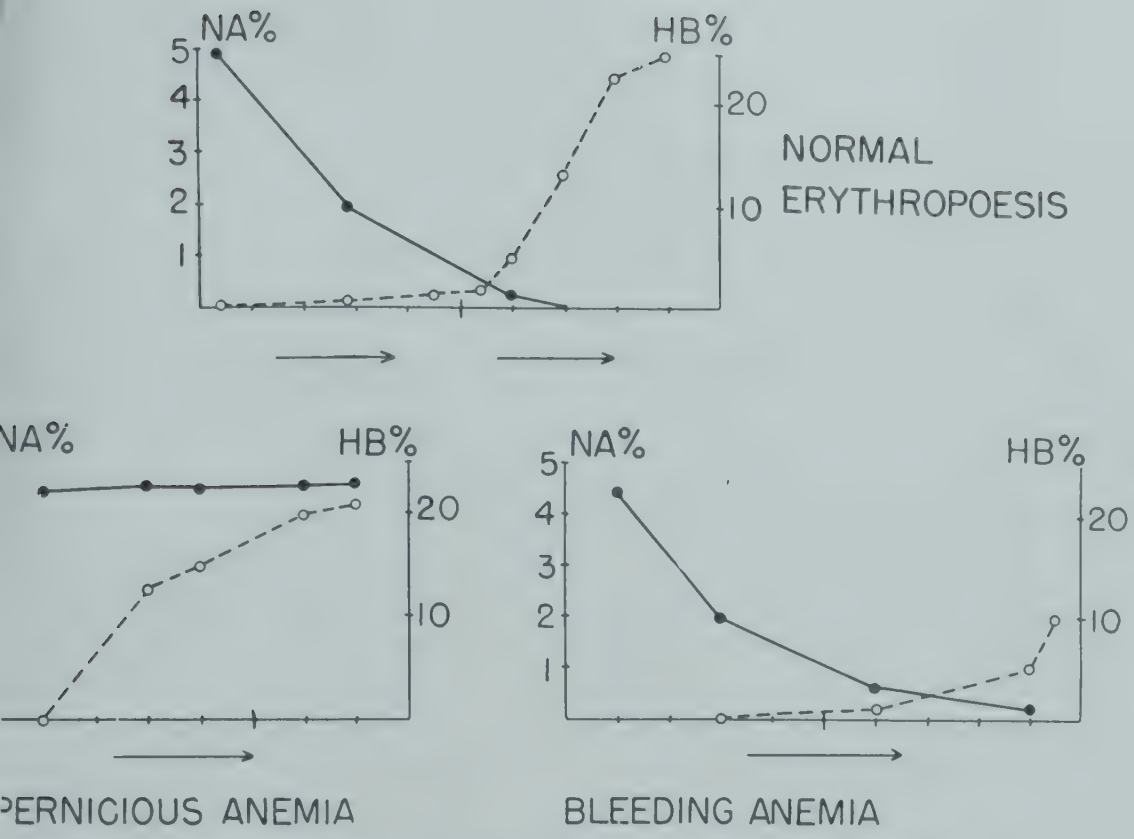


Fig. 59. Survey of the changes in the processes of endocellular growth and differentiation in the case of pernicious and hemorrhagic anemia. Abscissa as in fig. 44 and 48.
NA % = concentration of cytoplasmic ribose polynucleotides.
HB % = concentration of hemoglobin in the cytoplasm. See text.

The disturbances in the interplay between the growth and differentiation processes of erythropoiesis in the case of *hemorrhagic anemia* are of a different type. The decrease in the polynucleotide metabolism of cell growth proceeds normally, while on the other hand the hemoglobin synthesis in the cell exhibits a very low intensity.

Finally, the analyses show the possibility of attacking, with quantitative microspectrographical methods of analysis, certain disturbances in the relationship of the endocellular differentiation processes to the growth processes. The analyses also show that these disturbances may be looked upon as being specific for different types of pathological conditions during erythropoiesis, manifesting themselves as pernicious and hemorrhagic anemias respectively.

General Summary.

1. The purpose of the investigation is to analyse quantitatively the cytochemical processes involved in new formation and differentiation of the cellular substances during blood cell production.

2. A survey is given in Chapter 1 of the development of the cells of the blood in the adult.

3. The first part of the investigation deals with the *growth* processes during hematopoiesis. A starting point for this part of the investigation is provided by the experiences of the last ten years with respect to the endocellular ribose polynucleotide metabolism and its connection with the cellular protein-formation (Caspersson and coworkers). In Chapter 2 there is presented an account of this background work, and the primary problem of the investigation is formulated: does the new formation of cellular proteins during blood cell production proceed in the presence of *polynucleotides* with a cytochemical organization resembling that of other, previously investigated growing cells, *e.g.* embryonal cells.

4. An account is given of the methods and of the theoretical basis for cytochemical analyses of living bone marrow cells. The groups of substances which will be analyzed in the hematopoietic cells are: (1) the polynucleotides connected with the processes of cell growth; and (2) the specific substances connected with the differentiation processes, such as hemoglobin. The author describes in Chapter 3 two techniques for micro-spectrography: (1) a photographic method combined with photometry for use in the ultraviolet; and (2) a photoelectrical method for working in the visible spectral range. These methods permit of quantitative determinations of polynucleotides and hemoglobin respectively within single bone marrow cells in a living state. The amounts determable are of the order of magnitude of 10^{-6} μg.

After a description of additional cytochemical and cytological methods used in the investigation (ultraviolet microphotography, Feulgen's nucleal reaction, cytometry), an account is given of the

results of analyses of cellular material from normal hematopoiesis in mammals and man. Certain macrochemical analyses are also described (polynucleotide-phosphorus determinations in immature blood cells) which confirm the ultramicrochemical analysis data.

5. In connection with the primary problem of the investigation (see above 3.) Chapter 5 in Part I deals with the endocellular growth processes during hematopoiesis. By comparing the analysis of the distribution and concentration of the ribose polynucleotides in the hematopoietic cells with the quantitative cytology of the bone marrow, it is shown that *the new formation of cellular proteins during hematopoiesis takes place at an early stage of development in the presence of high concentrations* ($> 5\ \%$) *of ribose polynucleotides in the cytoplasm and nucleolar apparatus. During blood cell maturation the ribose polynucleotide-containing nucleolar mass and the concentration of cytoplasmic polynucleotides decrease continously to* $0 \pm 0.5\ \%$ *parallel with a declining growth activity of the cell.* It is also concluded that the results afford an alternative possibility of appraising the growth state of an individual blood cell by ultramicrochemical analysis of the composition of the cytoplasm and nucleolar apparatus.

6. By comparison of cytochemical and cytological data during blood cell maturation, the cytogenetic regulating mechanism of the endocellular growth processes during hematopoiesis was investigated and is reported in Chapter 6. It is shown that *the decreasing growth activity of the individual blood cell during maturation is regulated by a definite part of the chromatin, which during the interphase, can be defined as the nucleolus-associated chromatin.* In the telophase-interphase this chromatin proceeds directly from the gene-bearing chromosome material. In all probability the decrease in growth activity during the blood cell maturation is primarily caused by a reduced function of an unchanged amount of nucleolus-associated chromatin. Not until later, when the differentiation is far advanced, is this chromatin eliminated quantitatively.

7. Part II presents the results of an investigation of the relationship of the cellular growth processes to the specific cell differentiation processes, *i.e.* the relationship between the new formation of the cellular proteins and the changes of these proteins into their specific functional structures. Chapter 1 deals with *the synthesis of hemoglobin* during erythropoiesis. The hemoglobin content in single bone marrow cells is determined by absorption micro-

spectrography at the porphyrin absorption band (405 mμ), according to the method described in Chapter 3, Part I.

It is found that *the endocellular synthesis of hemoglobin does not start before the ribose polynucleotide metabolism* (associated with the new formation of the basic cellular protein-substances) *is finished — the latter process being measurable as a decrease of the cytoplasmic ribose polynucleotide concentration from 5 % to < 0.5 %. After this maturation-phase the main synthesis of hemoglobin begins, and the cellular content rises rapidly from an amount less than 2.10^{-6} μg up to 25.10^{-6} μg, the final value being 28.10^{-6} μg.* Thus *on a quantitative cytochemical basis the unipotent stem cell development normally occurs in several different phases, each of which is characterised by a particular cellular activity: 1. the phase of growth, 2. the phase of declining growth, 3. the differentiation phase and 4. the phase of declining differentiation.*

8. These principles applying to the new formation and differentiation of the bulk of the red blood cellular substance during erythropoiesis, are shown in Chapter 2, Part II to apply also to the production of granulocytes and dentine cells. This indicates that these principles for the processes of the development of the unipotent stem cell into a functionating tissue cell are of a general character.

9. Starting from the knowledge reached above about the cytochemical processes connected with the new formation and differentiation of the cellular substances during normal hematopoiesis, the question of whether disturbances in these processes can be demonstrated in certain pathological conditions of blood cell formation was investigated, and is reported in Part III.

In the case of pathologically increased growth (acute myeloic and lymphatic leukemia), there exists a measurable hypertrophy of the ribose polynucleotide metabolism associated with the new formation of cellular proteins. It is concluded that the effects were so great that the disturbances of growth in these conditions can be graded. (Chapter 1.)

In certain anemic conditions (pernicious and hemorrhagic anemia) the disturbances in the relationship between cellular differentiation processes and endocellular growth processes are measureable as an abnormal polynucleotide- and hemoglobin composition of the cytoplasm during the cell genesis. These effects appear to be specific for the different types of pathological disturbance. (Chapter 2.)

I desire to acknowledge my great indebtedness to Prof. T. Caspersson for the interest he has shown in these investigations and for his valuable advice and criticism.

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